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FAO
FOOD AND
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41/11

Residues of some veterinary drugs in animals and foods

Azaperone
Bovine somatotropins
Chlortetracycline, oxytetracycline and tetracycline
Dexamethasone
Diclazuril
Eprinomectin
Febantel, fenbendazole and oxfendazole
Gentamicin
Imidocarb
Moxidectin
Nicarbazin
Procaine benzylpenicillin
Sarafloxacin
Spectinomycin

WORLD
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Food
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Monographs prepared by the
fiftieth meeting of the
Joint FAO/WHO Expert Committee
on Food Additives
Rome, 17-26 February 1998

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TABLE OF CONTENTS

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Rome, 17-26 February 1998

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ABBREVIATIONS USED IN THIS DOCUMENT

ADI	-acceptable daily intake	µm	-micrometer
AUC	-area under concentration-Time curve	mg	-milligram
		min	-minute
Av.	-average	ml	-millilitre
b.i.d.	-twice a day	MR	-marker residue
BP	-British Pharmacopoeia	MRL	-maximum residue limit
Bq	-Becquerel (one disint/sec)	MRT	-mean residence time
BST	-bovine somatotropin	MS	-mass spectrometry
bw, BW	-body weight	n or No	-number
^0C	-degrees Celeius	na	-not analyzed, assayed or available
^{14}C	-radiolabelled Carbon		
C_{max}	-maximum concentration	nd, ND	-not detected
CAP	-chloramphenicol	NER	-non extractable residues
TCi	-microeurie (radioactivity)	ng	-nanogram
cm^3	-cubic centimeter	nm, NM	-not measured, if applicable
conc	-concentration		
CTC	-chlortetracycline	nm	-nanometer, if applicable
CV	-coeffieient of variation	NMR	-nuclear magnetic resonance
d	-day		
DPM, dpm	-disintegration per minute	NOEL	-no-observed-effect level
ECD	-electron capture detector	OTC	-oxytetracycline
e.g.	-for example	ppb	-parts per billion
ELISA	-enyzme labelled immunoassay	ppm	-parts per million
EP	-European Pharmacopoeia	r	-regression coefficient
eq or EQ	-equivalents	RIA	-radioimmunoassay
F	-female	RSD	-relative standard deviation
FDA	-Food and Drug Administration	SA	-Specific Activity
		s.o.	-subcutaneous
g	-gram	SD	-standard deviation
µg	-microgram	SEM	-standard error of mean
GC	-gas chromatography	sic	-correctly spelled
GI	-gastrointestinal	s.i.d.	-once a day
GLC	-gas-liquid chromatography	$t_{1/2}$	-half life
		t_{max} or T_{max}	-time for maximum
GLP	-Good Laboratory Practice	TC	-tetracycline
GVP	-Good Veterinary Practice	TLC	-thin layer chromatography
h	-hour	TMS	-trimethylsilyl derivative
^3H	-tritium	TR	-total residues
HPLC	-high performance liquid Chromatography	TRA	-total radioactivity
		TSD	-thermionic specific detection
i.e.	-that is		
i.m., IM	-intra muscular	UD	-unchanged drug
i.m.i.	-intra muscular injection	USDA	-US Department of Agrieulture
i.p., IP	-intra peritoneal		
i.v., IV	-intra venous	USP	-United States Pharmacopeia
k_{el}	-rate constant		
kg	-kilogram	UV	-ultraviolet
L or l	-litre	V_D	-volume of distribution
LC	-liquid chromatography	v/v	-volume/volume
LOD	-limit of detection	wt	-weight
LOQ	-limit of quantitation	w/v	-weight/volume
LSC	-liquid scintillation counting	WT	-withdrawal time
		%	-per eent
M	-molar or mole	>	-greater than
M	-male	<	-less than
max	-maximum	≤	-equal or less than
m/m	-mass/mass	≥	-equal or greater than

INTRODUCTION

The monographs on the residues of, or statements on, the 18 compounds contained in this volume were prepared by the fiftieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, 17-26 February 1998. JECFA has evaluated veterinary drugs at previous meetings, including the 12th[1], 26th[2], 27th[3], 32nd[4], 34th[5], 36th[6], 38th[7], 40th[8], 42nd[9], 43rd[10], 45th[11], 47th[12] and 48th[13] meetings.

In response to a growing concern about mass-medication of food producing animals and the implications for human health and internstional trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984[14]. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly-created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA[15]. In response to these recommendations, the thirty-second JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, the 34th, 36th, 38th, 40th, 42nd, 43rd, 45th, 47th, 48th and 50th meetiogs of JECFA were also dedicated exclusively to evaluation of veterinary drugs.

The teoth session of the CCRVDF, held in San José, Costa Rica, during October-November 1996, revised the priority list of veterinary drugs requiring evaluation. The drugs evaluated during the 50th meeting of JECFA included these compounds, except cyhalothrin, olaquindox and porcine somatotropin, the evaluation of which was postponed to a future meeting of the Expert Committee.

The present volume contains monographs of the residue data on 16 of the 18 compounds on the agenda. For the two compounds, azaperone and diclazuril, submitted for a toxicological re-evaluation, only a statement of confirmation of the existing maximum residue limits was made.

The anthelminthic agents, febantel, fenbendazole, oxfendazole and moxidectin had been coosidered before by the Committee. Eprinomectin, an anthelminthic agent, had not been evaluated before.

Five of the seven antimicrobial agents, chlortetracycline, oxytetracycline, tetracycline, gentamicin, and spectinomycin had previously been evaluated by the Committee. The remaining antimicrobial agents, procaine benzylpenicillin and sarafloxacin had not been evaluated before.

Of the three antiprotozoal agents,diclazuril had been previously evaluated by the Committee. Imidocarb and nicarbazin were before the Committee for the first time.

The remaining three compounds, dexamethasone, a glucocorticosteroid, bovine somatotropin, a production aid, and azaperone, a tranquilising agent, had been evaluated previously by the Committee.

The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the present 50th meeting is included in Annex 1.

The assistance of the experts and FAO consultant in preparing these monographs is gratefully acknowledged.

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AZAPERONE

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ADDENDUM

to the Azaperone monograph prepared by
the forty-third meeting of the Committee and published in
FAO Food and Nutrition Paper 41/7, Rome 1995

At its forty-third meeting the Committee had recommended temporary Maximum Residue Limits (MRLs) for azaperone residues in edible tissues of pigs expressed as the sum of the concentrations of azaperone and azaperol.

No new residue data were provided for consideration by the Committee. As the temporary MRLs established by the forty-third Session of the Committee are consistent with the good practice in the use of veterinary drugs, the Committee decided to delete the temporary qualification and confirmed the numerical values for MRLs in pig tissues as follows:

Muscle	60 µg/kg
Liver	100 µg/kg
Kidney	100 µg/kg
Fat	60 µg/kg

The Maximum Residue Limits are expressed as the sum of the concentrations of azaperone and azaperol.

BOVINE SOMATOTROPINS

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ADDENDUM
to the Bovine somatotropins monograph prepared by
the fortieth meeting of the Committee and
published in FAO Food and Nutrition Paper 41/5, Rome 1993

INTRODUCTION

The four analogues of bovine somatotropins somagrebove, sometribove, somavubove and somidobove that are produced by recombinant DNA-technology (rbST) were previously evaluated by the Committee at its fortieth meeting (WHO TRS 832, 1993, WHO FAS 31, 1993, FAO FNP 41/5, 1993). At that time the Committee established an ADI "not specified" for rbSTs. The term ADI "not specified" was used because of the lack of oral activity of rbSTs and of insulin-like growth factor I (IGF-I), as well as the low levels and non-toxic nature of the residues of these compounds, even at exaggerated doses, resulting in an extremely large margin of safety for humans consuming dairy products from rbST-treated cows. The Committee concluded that the use of these drugs according to good practice in veterinary medicine does not represent a dietary hazard to human health and that there was no need to specify a numerical ADI. Accordingly, no MRLs need to be set. In the meantime (1993), the Committee of Veterinary Medicinal Products of the EEC (CVMP) also concluded that the use of the rbSTs sometribove and somidobove in dairy cattle did not present any risk to the health of the consumers of meat and milk obtained from treated animals, and that due to their safety, the somatotropin products may be used without any withdrawal period for meat and milk. The CVMP considered that it is not necessary for the protection of public health to establish maximum residue limits (MRL) for rbSTs.

The recommendations of the 40th JECFA were deliberated by the Codex Alimentarius Commission, at its 22nd session in June, 1997. The Commission voted that the proposal to adopt the ADI and MRL proposal of "not specified" for rbSTs be postponed pending a reevaluation of new scientific data by JECFA at its 50th meeting in February 1998.

Information was submitted by organizations and individuals relating to the following concerns about the safety of the consumers of dairy products from rbST-treated cows:

- the increased use of antibiotics with a higher rate of violative drug residues in milk due to a possible increased incidence of mastitis in rbST-treated cows,
- the possibility that increased levels of IGF-I in milk of rbST-treated cows lead to increased cell division and growth of tumors in humans,
- the potential effect of rbST on the expression of certain viruses in cattle, particularly the retroviruses,
- the possibility that the incubation period of bovine spongiform encephalopathy (BSE) is shortened due to an IGF-I induced increase of the production of pathogenic prion proteins, and
- the possibility that early exposure of human neonates to milk from rbST-treated cows increases the risk for developing insulin-dependent diabetes mellitus.

BIOLOGICAL DATA

Use of antibiotics

The effect of rbST treatment to induce an increase of mastitis and somatic cell count in milk of treated cows was not reviewed by the Committee at its 40th meeting. These effects on animal health were considered outside the terms of reference of the Committee.

At its 50th meeting the Committee considered the literature data and the results of a post-approval monitoring program for sometribove (Posilac®) in the United States on the influence of rbST on mastitis and animal health. It was concluded that the effects of rbST on the incidence of mastitis and general animal health as well as the resulting days of treatment per animal with any medication are an issue of animal health and outside the terms of reference of the Committee.

However, the results of the post-approval monitoring program (PAMP) on the percentage of milk discard due to violative drug residue as a consequence of antibiotic use after the launch of Posilac® was considered by the Committee. The PAMP was initiated by the US FDA at the time of approval of sometribove (Posilac®) in November 1993 and started with its commercialization in February 1994. The objectives of this program were to determine whether mastitis incidence and antibiotic use was manageable under actual conditions of use, and whether label directions were adequate (FDA, 1996). The program was designed to address the following areas (Collier, 1996):

- the incidence of mastitis and responses related to herd health (not within the terms of reference of the Committee),
- the treatment with any medications in a 28-herd study with rbST-treated cows (not within the terms of reference of the Committee),
- to examine the incidence of milk discard due to violative drug residues in key dairy states representing at least 50 % of the U.S. milk production.

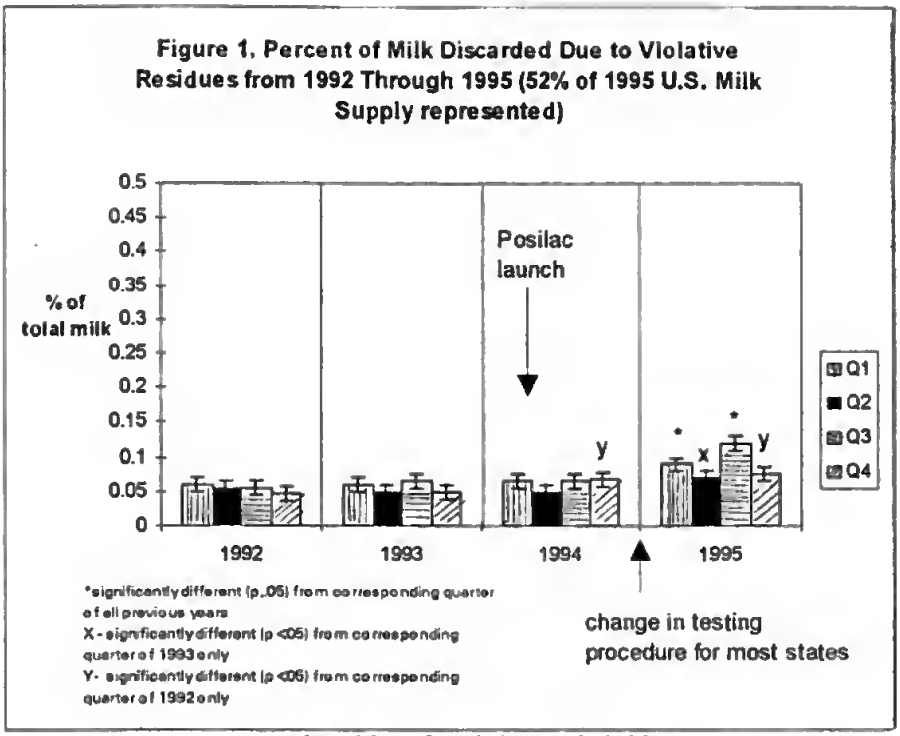
The PAMP was closely monitored by the FDA and performed according to the sponsor's Quality Assurance Standard Operating Procedures. The FDA confirmed that data integrity was acceptable and that data records and analysis showed excellent fidelity (FDA, 1996).

A program was designed for tracking milk residues by key dairy states before and after the approval of sometribove to reveal whether a possible increase of violative drug residues in milk is associated with increased frequency of use of antibiotics for mastitis and other health problems in rbST-treated herds of dairy cows (Veenhuizen *et al.*, 1996). The data from the milk monitoring program for the two years prior to the commercial use of sometribove (1992-1993) was compared to the discard data for two years after the launch to the market (1994-1995). The tracking of residues in milk was recorded by the National Drug Residue Milk Monitoring Program in which all bulk milk tanker trucks are routinely sampled and tested. The data set represented greater than 50% of the total U.S. milk supply. The data were analyzed quarterly by comparing the milk discarded prior sometribove launch with data after launch.

As seen in Figure 1, no change was observed in 1994 after Posilac® was approved. The average percentage of milk discarded was 0.06 % for 1992 as well as for 1993 and after launch 0.07 % in 1994. In 1995 the number of violations slightly, but significantly, increased to 0.09%. This increase, however, coincided with a change of the testing procedures in most states to include more sensitive screening tests in 1995 and that is believed to be responsible for the slight increase seen in 1995. Data reported by Veenhuizen et al. (1996), and submitted to FDA in the drug experience report of May 17, 1996 demonstrated that for New York State there was no significant change in milk discard rate for both years after approval of Posilac compared to the two years prior to approval. In New York, the same testing protocol had been in use throughout the entire four-year period (1992-1995). The values were 0.062% pre-approval and 0.064% post-approval. As reported by the Company launching sometribove, rbST was purchased by nearly 37% of the farms in the state, and these farms represented approximately 50% of the state's cows. These data indicate that:

- no product related increases in violative residues occurred in the years following commercialization of sometribove,
- the rate of positive tests is even slightly lower as compared with the monitoring results for antibiotics in Grade A milk in the U.S.,
- the use of sometribove will not have an impact on the safety of milk and dairy products due to violative drug residues resulting from a slightly higher medication rate in rbST-treated animals due to the procedures used by the milk monitoring program.

It was concluded that the use of rbST will not result in a higher risk to human health due to the use of antibotics to treat mastitis and that the increased potential for drug residue in milk could be managed by practices currently in use by the dairy industry and by following label directions for use (FDA, 1996).



adapted from Veenhuizen et al., 1996

bST and IGF-I levels in tissues and milk

Tissue levels of bST and IGF-I

A recent study by Choi *et. al.* (1997) reported the findings on the tissue levels of bST and IGF-I in cattle that had been treated with a 14-day sustained release product containing the natural variant of rbST, somavubove. The authors ran two experiments, A and B. Experiment A was comprised of three groups (12 animals per group, except the low dose group where only 6 animals were employed) of beef cattle whose average weight was 450 kg. The animals were treated by subcutaneous injection for 20 weeks. The controls received no treatment or vehicle, whereas the low dose (LOW), and the high dose (HIGH) groups received 250 mg of rbST at one-week, and 500 mg of rbST at two-week intervals, respectively. It was noted that the control and high dose groups were further divided into a low- and high-energy feed. The stated total dose was 5 and 10 grams of rbST, respectively; however, the dose calculated from the stated regimens would be 5 grams for both groups. Two weeks after final treatment, the animals were slaughtered and muscle samples were obtained and stored at -20°C for analysis.

In the second experiment (B), four groups of beef cattle were employed. They were a control group (CONT) that did not receive any drug or vehicle, a sustained-release low-dose (SR-L), a sustained-release medium-dose group (SR-M), and a sustained-release high-dose group(SR-H). The drug treated groups were administered the drug by s.c.-injection every two weeks for 24 weeks as follows: SR-L, 0.42 mg rbST/kg b.w. (0.03 mg/kg b.w./day); SR-M, 0.84 mg rbST/kg b.w. (0.06 kg b.w./day); and SR-H, 1.26 mg/kg b.w. (0.09 mg/kg b.w./day). The total dose was 2.3 g, 4.5 g, and 6.8 g for the respective dosing regimens. Animals were slaughtered and samples of muscle, kidney, liver and fat were taken two weeks following the final treatment and again stored at -20°C.

Frozen samples were assayed for bST and IGF-I residues by radioimmunoassay (RIA) procedures. The assays employed five grams of tissue and used acid ethanol for the extraction of muscle, and acetic acid for extraction of kidney, liver, and fat samples. The RIA procedures used standard double antibody techniques and iodinated tracers. The detection limit for the assays (the amount that could be distinguished from zero concentration with 95% confidence) was 0.17 ng/g and 0.61 ng/g for bST and IGF-I, respectively. Coefficients of variation for the two assays were approximately 6% or less, and

recoveries in liver, kidney and fat were 64.4% and 84.3% for bST and IGF-I, respectively. Similar recoveries were obtained in muscle samples. A summary of the results are seen in the following Tables 1 and 2.

Table 1. bST Levels* in Tissues after rbST Treatment

Experiment A	Tissue	CONT	LOW[1]	HIGH	
	Muscle (n=12)	1.87 ± 1.82 (a)	1.55 ± 1.62 (a)	3.25 ± 2.17 (a)	
Experiment B		**CONT**	**SR-L**	**SR-M**	**SR-H**
	Muscle (n=5)	3.38 ± 1.51 (a,b)	4.94 ± 1.47 (b)	3.78 ± 1.96 (b)	1.47 ± 0.86 (a)
	Fat (n=4)	5.05 ± 1.67 (a)	9.33 ± 5.23 (a)	4.82 ± 1.95 (a)	11.24 ± 11.95 (a)
	Liver (n=4)	5.18 ± 0.59 (a)	3.56 ± 1.73 (a)	5.36 ± 1.21 (a)	4.63 ± 1.96 (a)
	Kidney (n=4)	3.58 ± 1.14 (a)	4.45 ± 1.62 (a)	4.49 ± 1.83 (a)	3.92 ± 0.94 (a)

1 -number of animals in LOW group = 6; * levels in ng/g are expressed as the mean ± SD;
a and b -the same letter means that there is no statistically significant difference between values

Table 2. IGF-I Levels* in Tissues after rbST Treatment

Experiment A	Tissue	CONT	LOW[1]	HIGH	
	Muscle (n=12)	88.1 ± 21.0 (a)	131.8 ± 24.6 (a)	115.4 ± 32.1 (a)	
		(a)	a	a	
Experiment B		**CONT**	**SR-L**	**SR-M**	**SR-H**
	Muscle (n=5)	44.5 ± 6.5(ab)	34.9 ± 15.2 (b)	39.7 ± 5.1 (b)	54.5 ± 18.5 (a)
	Fat (n=4)	210.2 ± 84.8 (a)	204.3 ± 64.6 (a)	203.6 ± 52.6 (a)	339.1 ± 229.2 (a)
	Liver (n=4)	349.7 ± 23.5 (a)	389.6 ± 132.3 (a)	383.9 ± 168.1 (a)	294.4 ± 88.4 (a)
	Kidney (n=4)	913.5 ± 133.5 (a)	997.0 ± 140.2 (a)	821.1 ± 124.0 (a)	979.4 ± 219.4 (a)

1 -number of animals in LOW group = 6 * levels in ng/g are expressed as the mean ± SD
a and b -the same letter means that there is no statistically significant difference between values

The authors conclude that two weeks after administration of rbST for extended times in two dosage forms and at two or three levels, the tissue concentrations of bST and IGF-I are not significantly different from untreated control animals.

IGF-I residues in milk

Information on the residues of bST and IGF-I residues in milk of rbST-treated cows was evaluated by the Committee at its fortieth meeting (FAO FNP 41/5, 1993).

In bovine milk IGF-I is a normal, but highly variable constituent with the concentration depending on the state of lactation, nutritional status, and age. Over an entire lactation the IGF-I levels range from 1-30 ng/ml with highest concentrations in colostrum followed by constant decline thereafter. Multiparous animals have higher IGF-I concentrations in milk than primiparous (first calf) cows (Burton *et al.*, 1994). Bulk tank milk from cows not receiving rbST had IGF-I concentrations ranged from 1 to 9 ng/ml (Juskevich and Guyer, 1990). The JECFA monograph on BST (FAO FNP 41/5, 1993) cited average control values of 3.7 ng/ml for untreated cows. In milk from rbST-treated cows the levels of IGF-I ranged from 1-13 ng/ml in most studies and were about 25-70% greater as compared with untreated animals (Burton *et al.*, 1994). The JECFA monograph reported average IGF-I concentrations of 5.9 ng/ml (FAO FNP 41/5, 1993). The increase was significant even though most of the measured IGF-I levels were below 10 ng/ml.

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Since the original reported work was reviewed, very little additional residue data has appeared in the literature or in reports made available by sponsors. Monsanto, manufacturer of POSILAC® - previously identified as sometribove which is one of the forms of rbST approved in a number of countries - submitted additional information on levels of insulin-like growth factor-1 (IGF-I) in milk. The study (Eppard *et al.*, 1994) was designed to determine the levels of IGF-I in retail milk samples and to compare IGF-I levels in milk which was specifically labeled that it did not come from bST treated cows with IGF-1 levels in milk which was not labeled. While the sponsor assumes that the unlabeled milk contained milk from cows treated with bST, the extent to which herds contributing to the unlabeled milk were treated with bST was not ascertained. The study was conducted under FDA's GLP regulations (21CFR 58).

The labeled and unlabeled status of 127 of 129 retail milk samples (4 goats and 125 cows) was determined and subjected to analysis by radioimmunoassay (RIA) for IGF-1. Seventy eight samples were labeled as farmer certified that rbST had not been used. As indicated above, the remainder of the samples did not identify any absence of treatment and were inferred by the sponsor to include herds from rbST treated cows. The samples were collected from retail outlets as 2%-fat cow's milk in 34 cities located in Wisconsin, Minnesota, and Iowa. Fifty-one brands and 51 stores were represented. Note that rbST is not approved for use in goats in the United States and is therefore assumed to reflect untreated IGF-1 values for this species. The results are shown in Table 3.

These values for IGF-I are not unlike those reported in the first JECFA monograph on BST (FAO FNP 41/5, 1993). Even though it is not known to which extent milk from rbST-treated cows contributed to the unlabeled milk, the data indicate that in the first year after launch of rbST the IGF-I concentrations in retail milk did not increase.

Table 3. Insulin-like growth factor-I (IGF-I) concentrations in milk identified as farmer certified that rbST was not used (labeled) and non-labeled milk.

	SAMPLE		
	Labeled (ng/ml)	Non-labeled (ng/ml)	P-value
Raw mean	4.3 ± 0.09	4.5 ± 0.12	
Log_e[1]	1.47 ± 0.044	1.55 ± 0.031	0.1769
Antilog (95% confidence interval)	4.4 (4.0, 4.7)	4.7 (4.4, 5.0)	

[1] n = 78 labeled, 45 non-labeled samples. Least square means adjusted for state where purchased and dairy brand.

Assay values

Because of variations of IGF-1 values in different studies, questions have been raised in submissions to the JECFA regarding the accuracy of the IGF-1 milk values. In some studies lower values were measured because they were obtained by an assay that used acid ethanol extraction. The availability of another assay which employs acidic gel filtration has been quoted as achieving superior recovery when compared to the acid ethanol procedure. Vega *et al.* (1991) have reported on

the difference in these two assays when determining the IGF-I in pre- and postpartum mammary secretions. These authors calculated that the acid ethanol assay underestimated IGF-I levels by 24 ± 6.6 % when comparing values obtained with acid gel filtration. The problem is that the IGF-I binding protein which has a respectable binding affinity for IGF-I competes with the antibody used in the RIA procedure; however, the gel filtration removes the hormone when it extensively dissociated by the acid that is common to both assays. Studies on the acid ethanol assay by Hadsell (1991) examined the recovery of ^{125}I-IGF-I in bovine serum and colostrum which also contain binding proteins. The author obtained values of 86 ± 6% and 88 ± 7%, respectively when testing the amount of label recovered by the antibody. These studies suggest that the acid ethanol assay probably underestimates milk and plasma values by 15-25 %. It is also apparent that if we are examining the relative difference in milk, the difference will probably not affect the outcome of any decision based on these low levels, although higher control values may affect the inferences being made. Though incomplete removal of IGF-binding proteins or variation of standard source might influence reported results, the Committee considered these factors not to materially alter any conclusions.

Bioavailability and bioactivity of IGF-I residues in milk

At its 40th meeting, the Committee concluded that many of the physiological effects of rbSTs are mediated by bovine insulin-like growth factor I (IGF-I) which is structurally identical to human IGF-I (WHO TRS 832, 1993). It was noted that there is a substantial synthesis of IGF-I mainly in the liver but it is also present in human milk, saliva and pancreatic secretions. It was further concluded that IGF-I had no bioactivity when administered orally to normal and hypophsectomized rats at doses up to 2 mg/kg bw/day. The role of dietary IGF-I was evaluated with the result that it is degraded by digestive enzymes and not active in the upper gastrointestinal tract.

Concerns have been expressed that a broad use of rbSTs in dairy production would lead to a sustained increase of the levels of IGF-I in bulk cow's milk and that the higher exposure of consumers would cause adverse health effects, if IGF-I survives digestion (Hansen *et al.*, 1997).

For a quantitative risk assessment the slight increases of IGF-I in milk of rbST-treated cows have to be compared with the physiological variations of this growth factor during lactation as well as with the levels in human breast milk, in the secretions of the gastrointestinal tract, and in serum.

In human milk the concentrations of IGF-I range from 8-28 ng/ml in the colostrum and from 5-10 ng/ml thereafter (Zumkeller, 1992, Burton *et al.*, 1994) indicating that breast-fed human neonates are normally exposed to IGF-I levels equal or higher as compared with milk of rbST-treated cows

Assuming a daily intake of 1.5 l of milk from rbST-treated cows with an average IGF-I concentration of 6 ng/ml the ingested amount of IGF-I is 9000 ng/day. The additional daily ingestion of IGF-I as compared with milk from untreated animals with an average IGF-I level of 4 ng/ml or 6000 ng/1.5 l would be 3000 ng.

By ingestion of milk from rbST-treated cows the slightly increased IGF-I levels contribute to the endogenous levels of IGF-I in the gastrointestinal tract of the consumers. The major site of IGF-I production is the liver in animals and humans. This peptide is further produced in the human gastrointestinal mucosa and is also found in saliva, bile, and pancreatic juice (Olanrewaju *et al.*, 1992). Chaurasia, et al. (1994) have determined the secretion of IGF-I in human gastrointestinal secretions. The five secretions and their average concentrations of IGF-I are as follows: saliva, 0.9 nM; gastric juice, 3.5 nM; jejunal chyme, 24.6 nM; pancreatic juice, 3.6 nM; and bile, 0.9 nM. Using a molecular mass of 7.5 kD for IGF-I (Zumkeller, 1992) and the volume for production of each of the fluids (Vander et al., 1990) permits calculation of the total production for IGF-I emptying into the gastrointestinal tract at 383,000 ng/day. The following Table 4 uses the results of the concentration data, along with daily production values for the various secretions and shows the calculated values for total IGF-I secreted.

The data in Table 4 indicate that the amount of endogenous IGF-I emptying into the gastrointestinal tract on a daily basis is more than (383000/9000) 42 times greater than the amount present in 1.5 liters of milk of rbST-treated cows. The 9000 ng value is 2.3% of the estimated daily gastrointestinal secretion of IGF-I in the adult. The additional daily ingestion of IGF-I of 3000 ng as compared with milk from untreated animals represents 0.78% of the gastrointestinal secretion.

Table 4. **Gastrointestinal secretion of IGF-I from various sources in the gastrointestinal tract.**

	Volume[1]	Concentration	Total mass of IGF-I
Secretion	ml/day	average ng/ml	secreted in ng
Jejunal chyme	1500	184.5	276750
Pancreatic juice	1500	27.0	40500
Gastric juice	2000	26.2	52400
Bile	500	6.8	3400
Saliva	1500	6.8	10200

[1] Vander *et al.* (1990) after Bauman, 1995

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Based on recent studies discussed below, it is postulated that, in contrast to the previous conclusion of the Committee (WHO TRS 832, 1993) of a complete and rapid degradation of IGF-I in the gastrointestinal tract, milk-borne IGF-I may partially escape digestion by proteases. It may, therefore, be bioactive in the intestine (Hansen *et al.*, 1997), or even be absorbed as intact peptide into systemic circulation (Epstein, 1996). In a study designed to investigate the potential of IGF-I peptides as therapeutics in the gut and to check the possibility of orally active formulations the degradation of IGF-I in various segments of the gastrointestinal tract of the rat *in vivo* and *in vitro* was determined (Xian *et al.*, 1995). Compounds that reduce the rate of degradation were also studied. The authors employed ^{125}I labeled IGF-I and monitored the extent of degradation by three methods. These included receptor binding, immunoprecipitation, and trichloracetic acid (TCA) precipitation. The model used two gut segments from each anaesthetized male Sprague-Dawley rat that had been fasted for 24 hours. Ligated segments of duodenum, and ileum, or whole stomach, and part of the colon were used. A bolus of labeled IGF-I (8.6 ng/ml in 0.2% BSA w/v saline) was injected into each segment and incubated for various times up to 60 minutes. The reactions were stopped and the flushed luminal contents were examined for the intactness of the labeled IGF-I by the three methods. The parallel set of *in vitro* experiments utilized flushed luminal contents from each of the four gut segments as a source of degredation enzymes. The results are found in the following Table 5.

Table 5. **Half-life of intact ^{125}I-labeled IGF-I in ligated Sprague-Dawley Rat gut segments (A) and in in-vitro flushings (B)**

Test for Intactness	Duodenum/Ileum		Stomach		Colon	
	A	B*	A	B*	A	B*
TCA	2 min	2 min	8 min	50 min	38 min	>60 min
Ab binding	2 min		5 min		33 min	
Receptor binding	2 min	2 min	2.5 min	3 min	16 min	ND

* for *in vitro* values (B), Ab and membrane receptor values reported as receptor binding; ND = not done

The data show the most rapid degradation is encountered in the duodenum and ileum segments and in their flushings (in vitro, B) followed by the stomach and then by the colon. In all cases the *in vitro* values were equal to or greater than the *in vivo* values.

The authors also examined the effectiveness of slowing the degradation rate of IGF-I in the gut by protecting the molecule in several ways. Among those tested, casein was the most protective exhibiting >90% protection in both the TCA and receptor assays in stomach flushings at concentrations of 10 mg/ml. In duodenal flushings, casein exhibited 80% (TCA

assay), but only 36% (receptor assay) protection against IGF-I degradation when the maximal casein concentration of 40 mg/ml was employed. The half-life of IGF-I (measured with the receptor assay) in the upper gastrointestinal tract increased from 2-3 min (Table 5) in the absence of casein and to up to 35 min in its presence.

This experiment appears to demonstrate a significant amount of protection by casein, at a concentration to the levels of this protein in milk. However, the authors acknowledge that the observed effects can be explained by the simple argument that there is competition by the additional proteins for degradation by the proteases in the respective segments. The experiment demonstrates that even with a high amount of protection from the protease activity, biological receptor binding activity, which is the best indicator of biological activity, is dramatically reduced.

These results were interpreted with regard to milk residues of IGF-I that "the protective effect of casein makes irrelevant the argument that human saliva contains IGF-I at levels greater than the quantities that would be consumed in milk. As the IGF-I produced by salivary glands is free IGF-I, without protective effect of casein, it is unlikely to survive digestion" (Hansen *et al.*, 1997). This argumentation neglects the following facts:

- Saliva is not the only source of IGF-I in the gastrointestinal tract. The majority is secreted in the gastrointestinal tract and the high concentration in intestinal chyme indicates that IGF-I is secreted in substantial amounts by the mucosa throughout the whole gastrointestinal tract (Olanrewaju *et al.*, 1992, Chaurasia *et al.*, 1994).
- Casein is flexible in its structure and is known to be readily degraded in the stomach and in the small bowel (Xian *et al.*, 1995). Thus, the protective effect will only be present in the upper gastrointestinal tract.
- The half-life of IGF-I in the presence of casein is only 35 min in the intestine (Xian *et al.*, 1995). Therefore, less than 5% of the initial IGF-I dose will survive more than 2 hours during the passage through the upper gastrointestinal tract.
- In the presence of casein ingested with milk the endogenous IGF-I in the gastrointestinal tract will also be protected.

It is therefore concluded that even considering a limited protective effect of casein the amount of bioactive IGF-I ingested with milk from rbST-treated cows will still be negligible.

Due to the protective effect of casein, some IGF-I might escape digestive degradation, being absorbed in intact form. A recent article by Kimura *et al.* (1997) studied the absorption of large oral doses of ^{125}I-rhIGF-I in a fasted adult rat model. Oral administration of 1 mg/kg doses of the labeled growth factor was followed by trichloroacetic acid precipitation of plasma proteins to evaluate the absorption of IGF-I. The baseline bioavailability of the administered IGF-I was determined to be 9.3% of the dose but was increased by co-administration of 4 mg/kg aprotinin (46.9%), and 10 mg/kg casein (67.0%). RIA analysis of the plasma further confirmed the bioavailability of IGF-I in this fasted rat model and the administered radioactivity was found in the form of high-molecular weight complexes. It should be noted, however, that the receptor assay which has the highest accuracy in measuring biological activity was not used.

The relatively large bioavailability of intact IGF-I in this adult rat model is not supported by the results of a lack of any oral bioactivity of IGF-I in adult animals (WHO TRS 832, 1993, WHO FAS 31,1993) as well as by the results of various studies with neonatal animals which have an incomplete mucosal barrier and a reduced intestinal proteolytic activity (Burrin, 1997). Studies in neonatal rats and piglets indicate that, although 30% of an orally administered dose of ^{125}I-IGF-I can be recovered in the intestinal mucosa, there is very limited absorption into peripheral circulation (Phillips *et al.*, 1995, Donovan *et al.*, 1997). In suckling transgenic rats, despite the ingestion of 1000-fold higher concentrations of des(1,3) human IGF-I, no des(1,3) IGF-I was detected in the plasma of the pups (Burrin, 1997). Furthermore, in studies with newborn calves and piglets given large doses of IGF-I in milk replacers no substantial increase of the plasma levels of this growth factor could be measured (Donovan *et al.*, 1997, Hammon and Blum, 1997, Houle *et al.*, 1997). In one study with newborn calves fed milk replacer, a small amount of orally administered ^{125}I-IGF-I could be detected in the blood plasma (Baumrucker *et al.*, 1992). However, the increase of plasma levels was not observed before three days of administration and was only detected in three of six animals. These results indicate that IGF-I even in neonates is absorbed to only a very small extent, and absorption is rather unlikely in adults.

Furthermore, the absorbed amount has to be compared with the normal levels of IGF-I in human serum which show considerable variation depending on age. The lowest values are observed in infants < 2 years, and constantly increase to reach a maximum in late pubertals and afterwards decrease to adult values as indicated in Table 6.

From the values in Table 6 and assuming a blood volume of 5% of the body weight (Ganong, 1971) a serum load of IGF-I of 49500 ng in a 15 kg child, 714000 ng in a 60 kg adult person and 1220000 ng in a 50 kg teenager can be calculated. The total IGF-I production in adults has been estimated as 10000000 ng per day (Guler *et al.*, 1989). These high amounts have to be compared with the IGF-I amount of 9000 ng in 1.5 l of milk, which constitutes only 0.09% of the daily IGF-I

Table 6. IGF-I concentrations reported in human blood plasma according to Schaff-Blass *et al.*, 1984

Age	Males [ng/ml]		Females [ng/ml]	
	Mean	Range	Mean	Range
0-2 years	42	14-98	56	14-238
3-5 years	56	59-210	84	21-322
6-10 years	98	28-308	182	56-364
Prepubertal > 10 years	126	84-182	182	70-280
Early pubertal	210	140-240	224	84-392
Late pubertal	364	224-462	434	224-686
Adult > 23 years	112	42-266	140	56-308

Total daily production of IGF-I in an adult = 10^7 ng/day (Guler *et al.*,1989)

production. Since only one third of the milk levels can be attributed to IGF-I caused by rbST treatment and only a very small amount if at all will be absorbed, the milk-borne IGF-I reaching systemic circulation is negligible and this small amount will be immediately sequestered by unsaturated binding proteins.

It can therefore be concluded that the slight increase of IGF-I in the milk of rbST-treated animals is many orders of magnitude lower than the physiological amounts of IGF-I produced in the gastrointestinal tract as well as in the body and will cause no relevant exposure of the consumer neither locally in the gut nor systematically.

Concerns have been expressed about possible adverse health effects in consumers exposed to increased IGF-I concentrations in milk from rbST-treated cows (Hansen *et al.*, 1997, Epstein, 1996). The most important potential adverse effects of IGF-I arise from the fact that it is a mitogen for a number of cell types and has been associated with the growth of various tumours including colon and breast cancer, osteosarcoma and lung cancer (National Institute of Health, 1995, McCaulay, 1992, Pines *et al.*, 1985). The mitogenic effect is further supposed to cause proliferative reactions locally in the gut. Thus, orally administered IGF-I to rats increased the in vivo cellularity of the intestinal mucosa (Olanrewaju, 1992) or increased the proliferation rate in cultures of human epithelial crypt cells from the duodenum (Challacombe and Wheeler, 1994). Since IGF-I receptors could be detected throughout the epithelium of the intestine and with a high density in the colon (Laburthe *et al.*, 1988) and the incidence of colorectal cancer is increased in acromegalic patients having excessively high levels of free IGF-I in the plasma (Ezzat and Melmed, 1991) concerns have been expressed that increased levels of milk-borne IGF-I may increase the risk of colonic cancer.

Although IGF-I has as a consequence of its normal biological effects the potential hazard to promote the growth of tumors, this hazard can only become a risk if there would be an adequate exposure of the consumers to increased amounts of IGF-I. Since the exposure to IGF-I by ingesting milk from rbST-treated cows is negligible when compared with the endogenous IGF-I production it is extremely unlikely that the IGF-I residues can cause any systemically or local adverse mitogenic reaction.

Expression of lentiviruses and prion proteins

Somatotropins and the immune system

Somatotropin (ST) has immunomodulatory effects. Immunoenhancing activity has been documented in many different species including cattle (Comens-Keller *et al.*, 1995). The primary effect appears to be altered responsiveness of the immune system even though data of a substantial nature of this effect are incomplete (Burton *et al.*, 1994). Information on changes in cytokine concentrations or secretion as well as their binding site populations are needed to define the nature of ST-immunoenhancing effects. The literature is inconsistent regarding the source of ST used, the ST treatment schedule and the age of the animals. Differences between *in vitro* and *in vivo* findings may be explained by ST-stimulated release of mediators in vivo such as IGFs and cytokines which are not present during in vitro studies (Kelley, 1989). A better understanding of ST-mediated immunoenhancement as homeorhetic regulation of the overall health and disease resistance of animals is needed. It has been reported that lymphocytes from rbST-treated cows have greater average maximum

lymphoblastogenic response to rbST as compared to other mitogens in the periparturient period (Comens-Keller et al., 1995). It is postulated that this effect might prove to be beneficial for prevention of mastitis or other infectious diseases that occur during the immunosuppressed periparturient period.

Effect of bST on the expression of retroviruses

Concerns have been expressed that the immunomodulatory effect of bST might effect retrovirus expression in treated animals and cause resurgence of latent retrovirus and lentivirus infections in the ruminant population and cause the occurrence of such viruses in somatic cells in milk. The concerns are largely based on a review by Lerondelle *et al.* (1994) discussing evidence of induction of these viruses in small ruminants by steroid hormones along with discussion of induction of lentiviruses in other species by other hormones including growth hormone and/or IGF-I and on an unpublished study of Lerondelle *et al.* (1996) who investigated the effects of rbST on the expression on Caprine Arthritis Encephalitis Virus (CAEV) in goats. This virus belongs to the group of lentiviruses which like Maedi/Visna can infect small ruminants.

There are at least three reasons why there might be interest in ruminant lentiviruses. First, they might be of concern to persons consuming the milk in that these viruses may cause illness in humans. Second, there may be additional concern that if the use of rbST causes increases in the ruminant viruses presumably through the presence of rbST itself, or the action of IGF-I, the presence of the small additional amounts of growth hormone present in milk of treated cows may also somehow affect the retroviruses which affect the human immune system known as HIV-1 and HIV-2. Finally, the potential to increase the severity or kinetics of expression of the disease in the ruminant itself.

The study by Lerondelle and coworkers (1996) attempts to address the last question as whether rbST increases the expression of Caprine Arthritis Encephalitis Virus (CAEV), a member of a family of retroviruses that infect small ruminants. Measurements of viral expression included assay of reverse transcriptase activity in cells in milk, a clinical examination of the udders and joints of animals at the beginning and end of the study, and evidence of infection by use of an immunodiffusion assay. Twelve pregnant Saanen goats, seronegative for CAEV were experimentally infected by treating them intramammarily with *in vitro* infected monocytes with the Cork strain of CAEV at the time of drying off. Groups of four goats were treated as follows beginning seven weeks after giving birth: one group treated with rbST (sometribove); a second group treated with thyroxine; and the control group was untreated. Doses of 5 mg/day/goat for rbST, and 10/mg/goat/day for thyroxine were administered in suspension in sterile water. The drugs were given for 30 days followed by a 45 day observation period. Milk samples were taken for reverse transcriptase activity on treatment days 7,14, 21 and 28, and three times during the observation period. As indicated previously, udder and joint health as well as immunodiffusion tests were also done at the beginning, and end of the study. In addition, milk production, and milk cell counts were evaluated every two days. The results shown in Table 7 were presented to describe the occurrence and onset of the appearance of CAEV viral effects.

The results show the time in days for the cultured milk cells to exhibit evidence of viral expression in the cells harvested at the designated milk sampling times. The data show that there is greater evidence of positive cultured cells from the control treatment than either of the hormone treatments. Perhaps the most striking effect is the lack of a positive increase in the rate of infectivity, and even the suggestion of a decrease in infected cells as seen in goats 9431 and 9436, as a result of the treatment with rbST in particular after the first milk sampling period.

Among the studies the authors carried out on the milk of the CAEV infused goats, the one shown in Table 8 examines the infectivity challenge and its attempted augmentation by treatment with the hormones, thyroxine and rbST. The test employed the reverse transcriptase assay and measures activity in cells considered positive for virus in culture and expressed as a ratio of transcriptase activity over number of positive cell cultures

The results of the study showed no positive correlation of the effect of rbST or thyroxine on the activity of reverse transcriptase in the milk samples as seen in the summary of results shown as Table 8. In fact, there appears to be no evidence of increased transcriptase activity in any of the groups. This is particularly interesting in the rbST group which appears to have a lower initial rate of infection than the two other groups including the controls, yet neither is there an increased rate of infection as measured by number of positive cultures nor is there an increase in transcriptase activity. The authors interpreted their results as a tendency of an increased virus expression with increased milk production. The results with rbST were, however, biased by the fact that only two animals were included in the final evaluation. The authors concluded that due to the heterogeneity of the effects on milk production and the small number of animals tested the data base for establishing the promoting effect of rbST on virus expression was insufficient to demonstrate a clear effect.

Table 7. Onset of appearance of the cytopathic effect (in days) for each of ten milk samplings from the control, thyroxine, and rbST groups as a function of the period of hormonal treatment.

Goats	Before Treatment			During Treatment				After Treatment		
Milk Samplings	1	2	3	4	5	6	7	8	9	10
Control										
9433	4	8	6	10	6	6	6	8	10	8
9434	4	6	6	6	8	6	6	6	-	10
9435	4	6	4	6	4	4	6	4	4	6
9439	6	8	10	6	10	6	-	-	-	-
M ± SD (n)	6 ± 1.91 (12)			6.4 ± 1.71 (15)				7 ± 2.39 (8)		
Thyroxine										
9430	8	6	10	4	6	6	-	-	6	4
9441	6	4	4	4	4	4	4	4	ND	4
9442	4	4	4	4	4	4	6	4	ND	4
9443	4	8	6	4	4	4	6	4	ND	4
M ± SD (n)	5.67 ± 2.06 (12)			4.53 ± 0.92 (15)				4.25 ± 0.71 (8)		
rbST										
9431	8	-	-	-	-	-	-	-	-	ND
9436	4	8	8	-	8	-	-	-	4	ND
9438	ND	-	-	-	-	-	-	-	-	ND
9440	4	4	4	4	4	4	4	4	4	4
M ± SD (n)	5.71 ± 2.41 (7)			4.8 ± 1.79 (5)				4 (4)		

Table 8. Number of positive samples by the Reverse Transcriptase-Positive Culture Ratio for the virus in the goats of control, thyroxine, and rbST treated goats.

Goats		Before treatment	During treatment	After treatment	Total
Control	9433	3/6	4/7	3/4	10/17
	9434	3/6	3/5	1/3	7/14
	9435	6/6	4/5	5/6	15/17
	9439	4/4	2/8	3/6	9/18
	Total	16/22	13/25	12/19	41/66
Thyroxine	9430	0/3	2/4	0/3	2/10
	9441	4/4	5/7	6/6	15/17
	9442	6/6	8/8	6/6	20/20
	9443	6/6	8/8	4/6	18/20
	Total	16/19	23/27	16/21	55/67
rbST	9431	2/4	0/6	0/1	2/11
	9436	6/6	7/8	2/5	15/19
	9438	0/2	0/4	0/3	0/8
	9449	4/4	4/5	5/6	12/14
	Total	12/16	11/23	7/15	29/52

These data provide no evidence that rbST treatment of cows infected with lentiviruses will cause resurgence of virus infections in ruminants or give rise to any risk of human health. Lentiviruses are a type of retrovirus which only replicate in activated immune system cells. They may stay dormant for months and years before they gradually wear down an immune system to the point of collapse. The phylogenetic tree of lentiviruses includes the subfamily of Bovine Immune Deficiency Virus (BIV) also called Bovine Leukemia Virus (BLV) as well as the subfamily of HIV-1 and HIV-2 virus which are causative agents of AIDS in humans. BIV and HIV are not the same viruses and are highly separated phylogenetically (Robertson, 1997). Furthermore, BIV is not known to cause disease in humans although the virus has shown the ability to infect human cells in vitro where host defense mechanisms would not be present (Georgiades *et al.*, 1978, Van Der Maaten and Miller, 1990). Infection of human cells in vivo could not be demonstrated. All attempts to obtain direct evidence of infection in exposed human populations have yielded negative results (Straub, 1981, Van Der Maaten and Miller, 1990). The failure of human infections has also been shown for other ruminant lentiviruses such as CAEV and Maedi/Visna (Straub, 1981). Excretion of virus with milk somatic cells can cause infection of the offspring of infected cows. This transmission can effectively be blocked by procedures similar to pasteurization of the milk which will destroy the virus at 60°C within 30 sec (Abramova *et al.*, 1974, Baumgartner, 1976, Van Der Maaten and Miller, 1990). Therefore pasteurization may also prevent the transmission of BIV to consumers of milk.

It is concluded that BLV cannot induce diseases in humans and is completely inactivated by routine pasteurization. Furthermore, according to a not further qualified statement of the company commercializing sometribove, there is no indication that the incidence of BLV has increased in cattle after 4 years of continuous use of rbST in the U.S. and 8 years in Mexico and Brazil (Collier *et al.*, 1998).

An increase of the expression of HIV-viruses in humans by ingestion of milk from rbST-treated cows is extremely unlikely due to the negligibly small residues of rbST and IGF-I. It has further been shown that treatment of AIDS patients for 6 weeks with recombinant human growth hormone and IGF-I had no influence on HIV levels associated with peripheral blood mononuclear cells, CD3, CD4, or CD8 counts in peripheral blood as well as serum HIV p24 antigen levels (Waters *et al.*, 1996).

Effect of rbST on Prion Proteins

Concerns have been expressed that rbST treatment could increase the risk of bovine spongiform encephalopathy (BSE) in dairy cows (Hansen *et al.*, 1997). Little evidence to support this concern has been provided, and that provided is indirect.

The present theory is that the infectious agent of BSE is a prion protein (PrPc) (Prusiner, 1982). PrPc's are normally found in all animals and are encoded by a prion-protein gene. BSE is associated with a post-translationally modified protease-resistant protein (PrPsc) which differs in its three-dimensional structure to the normal protease-sensitive PrPc. Normal PrPc's are found membrane-bound on the surface of all nerve cells, some lymphocytes and other tissues (Prusiner, 1991). To date no function has been ascribed to normal PrPc. The most widely accepted theory of BSE is the conversion of normal PrPc's to the abnormal PrPsc's form which in turn causes more normal PrPc's to convert to PrPsc's. The mechanisms of the conversion to the disease-causing PrPsc are not clearly understood. In contrast to the normal form, PrPsc cannot be turned over in cells, and builds up in the cell forming large oligomers observed as plaques (amyloids) in the brain of affected individuals (Gajdusek, 1993).

It has been demonstrated that IGF-I increases the production of PrP-mRNA in vitro in a rat phaeochromocytoma cell line (PC12 cells) with a rather flat dose response curve with a 40% increase at 10 ng/ml, and doubling at 100 ng/ml (Lasmézas *et al.*, 1993). In transgenic mice harbouring multiple copies of the PrP gene the speed of progession of Scrapie was increased (Prusiner, 1991). It has not been shown that IGF-I increases the formation of the PrPsc form of the protein, and thereby shortening the incubation period for BSE.

It is speculated that the increased IGF-I levels in rbST-treated cows would lead to an increased PrPc production and possibly speed up the progression of BSE. However, there are no data that directly address whether rbST or IGF-I increases the formation of normal PrPc or its pathogenic protease-resistant mutant in the brain of the cattle. Therefore, the possibility of a link between rbST treatment and BSE is highly speculative.

Cows milk and insulin-dependent Type 1 diabetes mellitus in childhood

In epidemiological studies performed in various geographical regions it could be demonstrated that among other environmental factors such as chemicals or virus infections the short duration of breast-feeding and the early dietary exposure of newborns to cow's milk containing formulas will increase the risk of insulin-dependent Type 1 diabetes mellitus (IDDM) by about 1.5 times (Scott, 1990, Dahlquist *et al.*, 1991, Jorgensen *et al.*,1991, Virtanen *et al.*, 1993 and 1994, Gerstein, 1994, Verge *et al.* 1994). IDDM develops as a consequence of autoimmune destruction of the insulin-producing ß-cells of the pancreatic islets The precise trigger of the autoimmune reaction is unknown (Gerstein, 1994). It is hypothesized to be a genetically acquired immune defect in susceptible individuals (Gerstein, 1994). Epidemiological evidence exists that IDDM is geographically and temporally related to neonatal feeding practice with cow's milk and that avoidance of cow's milk in the first few months of life can protect genetically predisposed individuals (Gerstein, 1994, Verge *et al.*, 1994).

Serological evidence supports the view that this immune defect may be triggered by exposure to proteins of cow's milk (Gerstein, 1994). It is postulated that in neonates, milk proteins may cross the immature gut wall initiating an immune response that crossreacts with a ß-cell surface antigen (Verge *et al.*, 1994). It could be shown that older children (5-9 years) with intact intestinal barrier are not at risk to acquire IDDM by exposure to cow's milk (Dahlquist *et al.*, 1991). The possible triggering factors in cow's milk have not been precisely identified. Casein seems to be unlikely since in diabetes-susceptible rats replacement of milk proteins by casein completely prevented diabetes (Jorgensen *et al.*, 1991). It is supposed that increased levels of IgA antibodies to cow's milk and beta-lactoglobulin are associated with increased risk if IDDM (Dahlquist *et al.*, 1991, Virtanen *et al.*, 1994). It is unlikely that exposure of human neonates to milk of rbST-treated cows increases the risk of IDDM for the following reasons:

- the composition of milk from rbST-treated cows is well within the normal variations observed during the course of lactation,
- the slightly increased IGF-I levels in cow's milk can be excluded as a triggering factor because of the identical nature of bovine and human IGF-I and that levels of IGF-I in breast milk are equal and initially higher than those found in cow's milk.

APPRAISAL

General

Information was submitted by organizations and individuals relating to the following concerns:

- the increased use of antibiotics with a higher rate of violative drug residues in milk due to a possible increased incidence of mastitis in rbST-treated cows,
- the possibility that increased levels of IGF-I in milk of rbST-treated cows might lead to increased cell division and growth of tumors in humans,
- the potential effect of rbST on the expression of certain viruses in cattle, particularly the retroviruses,
- the possibility that the incubation period of bovine spongiform encephalopathy (BSE) is shortened due to an IGF-I-induced increase of the production of pathogenic prion proteins, and
- the possibility that early exposure of human neonates to milk from rbST-treated cows increases the risk for developing insulin-dependent diabetes mellitus.

Use of antibiotics

After reviewing the data the Committee considered the risk of mastitis induced by rbST as an issue of animal health which is not within the terms of reference of the Committee. However, the possible increased use of antibiotics was considered.

A post approval monitoring program (PAMP) was established in the United States to address the following areas:

- the incidence of mastitis and responses related to herd health (not within the terms of reference of the Committee),
- the treatment with any medications in a 28-herd study with rbST-treated cows (not within the terms of reference of the Committee),
- the incidence of milk discard due to results from antibiotic residue testing in key dairy states representing at least 50 % of the U.S. milk production.

In New York State the percentage of milk discard resulting from antibiotic residue testing was not significantly changed after introduction of rbST. In other states a small, but statistically significant, increase was observed in 1995 which

coincided with a change to a more sensitive testing method. The Committee concluded that the use of rbST will not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for drug residues in milk could be managed by practices currently in use by the dairy industry and by following tabel directions for use.

IGF-I levels in milk and tissues

IGF-I is a normal component of milk and is found in abundance in variety of body fluids (see Table 9).

Table 9. IGF-I in milk and body fluids

Ftuid		[ng/ml]	Fluid		[ng/ml]
Milk	human	5 - 10	Gastrointestinal secretions (human)	Saliva	6.8
	human colostrum	8-28		Gastric juice	26
	bovine – untreated*	I - 9		Pancreatic juice	27
	bovine - rbST-treated*	I - I3		Bile	6.8
Plasma	child	I7 - 250		Jejunal chyme	I80
	adolescent	182 - 780	Daily production by adult humans = 10^7 ng/day		
	adult	123 - 460			

*bulk milk

The presence and concentrations of IGF-I were at the center of much of the scientific discussion in the original scientific review of bST undertaken by the 40th meeting of the Committee and in submissions to the present JECFA meeting. Information that was previously reviewed is summarized in FAO Food and Nutrition Paper No. 41/5 (1993). IGF-I concentrations in milk are variable and have been shown to depend on state of lactation, nutritional state, and age.

Methods for assaying IGF-I were considered by the Committee. Although incomplete removal of IGF-binding proteins or variation of standard source, and extraction methods might influence reported values, these factors were not perceived to materially alter any conclusions. Relatively high values previously reported in milk were considered to reflect inadequate extraction procedures.

Since the previous evaluation, very little additional data on residues have appeared in the literature and in reports provided by interested parties. However, the manufacturer of sometribove submitted additional information on levels of IGF-I in retail milk after the approval of rbST in the United States The results showed no difference in the IGF-I concentrations between labeled (certified to be derived from cows not treated with rbST) and unlabeled milk. However, the percentage of milk derived from cows receiving rbST was not specified for the unlabeled milk.

Concerns have been expressed that any rbST-induced increase of IGF-I in milk contribute to the endogenous levels of IGF-I in the gastrointestinal tract and in serum if not biodegraded, and if absorbed. A recent study in rats confirms that IGF-I is rapidly degraded in the gastrointestinal tract. However, in these studies a protective effect of casein on IGF-I could be demonstrated. It is postulated that the retarded degradation leads to increased serum levels of IGF-I (as has been shown in one study in rats) as well as to prolonged exposure of the gut as well as to increased serum levels of IGF-I. The Committee also noted that 7 days of oral administration of high doses of IGF-I in milk replacer did not increase circulating concentrations of IGF-I in newborn calves and piglets indicating that significant absorption of IGF-I is unlikely to occur under physiological circumstances in these food animals.

Considering the decreased rate of degradation observed in the small intestine in rats in the presence of casein, levels of the growth factor would likely deplete to less than 5% of their initial values within two hours indicating that milk-borne IGF-I would not be expected to contribute to levels of IGF-I in the large intestine.

Assuming the ingestion of 1.5 liters of milk per day, the average ingested amount of IGF-I will be 6000 ng for milk from untreated animals containing an assumed IGF-I concentration of 4 ng/ml and 9000 ng for milk of rbST-treated animals with an approximate average concentration of 6 ng/ml. It has been calculated that IGF-I in gastrointestinal secretions amounts to

about 380000 ng/day. Therefore, the additional amount of IGF-I in 1.5 liters of milk from rbST-treated cows as compared with milk from untreated cows is only about 0.8 % of the gastrointestinal secretion.

The total amount of IGF-I in serum has been calculated to range from approximately 50000 to 1220000 ng depending on age. The total daily IGF-I production in adult humans has been estimated as 10^7 ng. Therefore, the daily value of IGF-I ingested with milk from rbST-treated cows compared with the daily production will be less than 0.09% for adults. Even if the total amount of milk-borne IGF-I were absorbed the additional amount would be negligible.

The Committee concluded that any increase of IGF-I in milk from rbST-treated cows is orders of magnitude lower than the physiological amounts produced in the gastrointestinal tract as well as in other parts of the body. Thus, the Committee concluded that there will be no increased exposure of the consumers either locally in the gut or systemically. Consequently, the potential for IGF-I to promote tumor growth will not increase when milk from rbST treated cows is consumed, resulting in no appreciable risk for consumers.

Recent studies have been performed in which sustained release rbST was administered for 20 weeks. Tissue levels of of rbST and IGF-I were measured two weeks after the final administration of rbST. No significant increases in the rbST and IGF-I levels were observed.

Expression of retrovirus

Concerns that rbST treatment of cattle would increase the expression of retroviruses including Bovine Leukemia Virus (BLV), were addressed by experiments in a goat model that used caprine arthritis encephalitis virus. Infectivity was not increased when measured by numbers of infected cells, and there was no evidence of increased reverse transcriptase activity. These studies provided no evidence that rbST affects the expression of BLV, a lentivirus in cattle. Furthermore, it has been shown that BLV will be destroyed by simulated pasteurization conditions by heating milk to 60°C for 30 sec. In addition, there is no evidence of human susceptibility or responses to ruminant retroviruses.

Expression of prion proteins

Concerns have been expressed that rbST treatment could shorten the incubation period for bovine spongiform encephalopathy (BSE). This hypothesis is based on *in vitro* results in a neuronal cell line indicating an increased formation of mRNA of prion proteins (PrP) in response to IGF-I. Furthermore, in transgenic mice harbouring multiple copies of PrP gene, an increased formation of PrP shortened the incubation period of Scrapie. However, no data were available that directly address whether rbST or IGF-I increases the formation of normal PrP or its pathogenic protease-resistant mutant in the brain of cattle. The Committee considered that the possibility of a link between rbST-treatment and BSE to be highly speculative.

Risk of insulin-dependent diabetes mellitus (IDDM)

It has been shown, that exposure of neonates to cow's milk increases the risk of IDDM by about 1.5-fold. The Committee considered whether exposure of human neonates to milk from rbST-treated cows further increases this risk, and concluded that, because of its unchanged composition, the milk of rbST-treated cows is not expected to represent an additional risk to the development of IDDM.

On the basis of the following

- insignificant changes in quantities of milk discarded due to results from antibiotic residue testing after introduction of rbST into commercial use;
- low levels residues of rbST and IGF-I in milk,
- the degradation of IGF-I in the gut and its abundance in gut secretions;
- the extremely low levels of ingested IGF-I when compared to endogenous production;
- the lack of evidence that rbST stimulates expression of retroviruses,
- lack of information directly linking rbST-treatment and BSE; and
- the absence of significant changes in composition of milk from rbST-treated cows which may contribute to the additional risk of development of IDDM

the Committee concluded that rbST can be used without any appreciable health risk to consumers. The Committee reaffirmed its previous ADI and MRLs "not specified" for somagrebove, sometribove, somavubove, and somidobove.

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CHLORTETRACYCLINE, OXYTETRACYCLINE AND TETRACYCLINE

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At its 12th, 36th (Oxytetracycline only), 45th and 47th meetings of the Committee evaluated chlortetracycline, oxytetracycline and tetracycline and recommended at its 47th meeting the following Maximum Residue Limits (MRLs), expressed as parent drug, singly or in combination: 100 µg/kg in muscle; 300 µg/kg in liver; 600 µg/kg in kidney of cattle, pigs, sheep and poultry, and 100 µg/L in cattle and sheep milk, and 200 µg/kg in egg (poultry). An MRL of 100 µg/kg for oxytetracycline in muscle of giant prawn was also recommended. The Committee evaluated the analytical methods for measuring residues at these MRLs.

At previous Committee meetings the limiting factor in setting of the MRLs was the low value of the ADI, 0-3 µg/kg of body weight. As a result some of the MRL values were close to the limit of quantification (LOQ) of the methods available. However, this practical approach based on the limitations of the analytical methods resulted in theoretical maximum daily intake values 30% higher than the ADI.

With the new assignment of an ADI of 0-30 µg/kg of body weight, ten-fold higher than the previous ADI, the Committee recognised that the constraints placed on the recommended MRL assignments no longer exist. In particular, the LOQ of the currently available methods for tissues that have been performance tested would permit a satisfactory control of residues at twice the value of the previously established MRL. The analytical methods for monitoring tetracyclines in milk have LOQ values (15 µg/L) considerably lower than the present MRL set for milk. The Committee, therefore, considered rising the MRLs in tissues and milk.

The Committee recommended doubling the MRL values in edible tissues. The resulting MRLs are consistent with available methods and with good practice in the use of veterinary drugs. The Committee did not have information on the concentration of tetracyclines in milk that would interfere with the production of milk products such as yoghurt. The Committee, therefore, recommended no change in the MRL for milk.

The Committee recommended MRLs for chlortetracycline, oxytetracycline and tetracycline, expressed as parent drug, alone or in combination, as follows:

Muscle	200 µg/kg	for cattle, pigs, sheep and poultry
Liver	600 µg/kg	for cattle, pigs, sheep and poultry
Kidney	1200 µg/kg	for cattle, pigs, sheep and poultry
Eggs	400 µg/kg	for poultry
Milk	100 µg/L	for cattle and sheep

At its 36th meeting the Committee recommended for oxytetracycline an MRL of 100 µg/kg in muscle for all species. Fish was one of the species for which residue depletion data had been provided. At the present meeting the Committee recommended extrapolating this MRL in fish to 200 µg/kg. It further recommended that this MRL should remain temporary until data on the use patterns of oxytetracycline in aquaculture can be evaluated.

At its 47th meeting the Committee recommended an MRL of 100 µg/kg for oxytetracycline in muscle of giant prawn (*Penaeus monodon*). In view of available residue depletion data and the substantial increase in the ADI, the Committee recommended a full MRL of 200 µg/kg for oxytetracycline in muscle of giant prawn, expressed as parent drug.

The MRLs recommended above will results in a theoretical maximum daily intake of 370 µg of residues.

DEXAMETHASONE

First Draft prepared by
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Beit Dagan, Israel

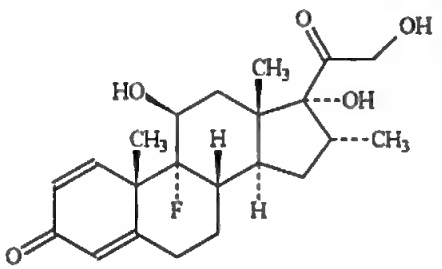
ADDENDUM

to the Dexamethasone monographs prepared by the forty-second and forty-third meetings of the Committee and published in FAO Food and Nutrition Paper 41/6, Rome 1994, and 41/7, Rome 1995, respectively

IDENTITY

Chemical Name: (11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione

Structural formula:



Active Ingredient: Dexamethasone

INTRODUCTION

Dexamethasone is a fluorinated glucocorticoid and a potent anti-inflammatory agent used frequently for treatment of inflammatory processes and primary ketosis in domestic food producing animals. Dexamethasone lacks effects on electrolyte balance but is 30-35 times more potent than cortisol as a anti-inflammatory agent.

At its 42nd and 43rd meetings the Committee reviewed dexamethasone (Wells, 1994a,b) and set temporary maximum residue levels (MRLs) of 0.5 μg/kg in muscle, 0.5 μg/kg in kidney and 2.5 μg/kg in liver of cattle, horses and pigs and 0.3 μg/L in cattle milk based on an ADI of 0 – 0.015 μg/kg. Dexamethasone can be administered to animals as the parent drug, or as one of several commercially available esters. Large variations in the absorption of the ester derivatives were reported. However, the esters are rapidly hydrolyzed in the blood to dexamethasone and dexamethasone was, therefore, considered the marker residue in terms of tissue residues. Considerable metabolism of dexamethasone was demonstrated. Apparently the metabolites do not carry any biological activity and, consequently, the parent drug, dexamethasone, was proposed as a marker residue.

In the JECFA review analytical methods for detection of dexamethasone at the set MRLs were thoroughly reviewed. It appeared that only the HPLC-MS method of the sponsor appeared to meet the criteria required for a residue control method at the allocated MRLs. However, the sponsor failed to provide full documentation of this method at that time. The dexamethasone analytical method was scheduled for review at the 48th meeting of the Committee but no data were received for evaluation. The Committee decided to withdraw the temporary MRL values set for dexamethasone due to lack of analytical method allowing enforcement of the set MRLs.

The present evaluation concerns only the documentation for the HPLC-MS method for control of dexamethasone residues in tissues and milk (Cook and McCormack,1996; Curl and McCormack,1996), provided by the sponsors Boeringer Ingelheim Vetmedica GmbH and Intervet International B.V.

General

The essential studies were performed in accordance to GLP. The appropriate references and statements were provided.

Sample preparation

Tissue, milk and serum sample preparation was performed using liquid/liquid extraction. The procedure appeared relatively simple and not too time consuming. The sample (5 g) was homogenized in 10 mL of Sorensen buffer. After centrifugation, the supernatant was extracted against hexane. Sodium hydrogen carbonate was added to the aqueous phase which was then extracted with 70% ethyl acetate in hexane. The extraction was repeated and the organic phases combined, evaporated to dryness and reconstituted in 0.5 N sulfuric acid. After hexane wash of the sulfuric acid fraction, sodium hydrogen carbonate was added to the aqueous phase. Extraction with 70% ethyl acetate in hexane was performed and the organic phase collected, evaporated to dryness and reconstituted in 50% acetonitrile in water. The sample was now ready to be injected to the chromatographic system.

Chromatographic method

The chromatographic method was based on gradient elution using an ODS2 (5 micron, 15 cm x 4.6 mm) reversed phase column. The mobile phase consisted of acetonitrile and 0.1 M ammonium acetate in ratio changing from 10:90 to 80:20 during the 10 minute chromatographic run. The injection volume was 100 µL.

Mass spectrometry

The Detection of dexamethasone was performed by thermospray mass spectrometry utilizing filament ionization. The ion source was adjusted at 230 °C and the initial probe temperature of 105 °C decreasing to 86 °C at 10 min was used. Single ion detection was employed and dexamethasone was monitored at 333 m/z and the internal standard (methyl prednisolone) at 315 m/z.

Quantitative calculations

Dexamethasone concentration in the sample was calculated by applying the detector response to a linear regression curve. The method uses internal standard (I.S.) for quantification because of large variation in detector response. However, it is not clear how peak area ratios (sample/I.S.) should be applied to the regression equation (Curl and McCormack, 1996). The amended report (Curl and McCormack, 1996) indicates that a subtraction of concentrations found in control samples should be done to the fortified samples. It is not clear how this procedure should be applied to incurred residue samples. The original report (Cook and McCormack, 1996) does not indicate how quantitative results were calculated.

Specificity

The presence of dexamethasone in the sample was determined on basis of retention time and typical MS ions. The ion 333 m/z in dexamethasone spectrum was observed to have highest abundance and was chosen as the ion to be monitored. Accordingly, the ion 315 m/z was chosen for the internal standard, methyl prednisolone. Of the compounds tested prednisolone, cortisone, methylprednisolone, triamcinolone, flumethasone, and isoflupredone did not interfere the dexamethasone analysis. However, betamethasone was found to elute together with dexamethasone and possessed the same 333 m/z ion and, consequently, it was not possible to distinguish between these compounds. The chromatograms provided with the amended report (Curl and McCormack, 1996) showed some apparent retention time instabilities. However, the report did not offer any explanation for this phenomenon.

Method validation

Linearity of the detector response was determined using concentration standards in the range from 0.25 to 10 ng/ml for milk and 0.5 to 20 ng/g for tissues and plasma. Linearity was considered to be acceptable when correlation coefficient exceeded 0.98. The recovery and accuracy of the method were determined by fortifying 5 replicates at concentration

Amended protocol

An amendment to the original report has been released (Cuel and MacCoemack, 1996). The purpose of the amendment was to comply with the EU guidelines and to the ISO 78/2 format. No essential changes concerning the method performance were added.

APPRAISAL

At its 42nd and 43rd meetings the Committee reviewed dexamethasone and recommended temporary maximum residue levels (MRL) of 0.5 µg/kg in muscle, 0.5 µg/kg in kidney and 2.5 µg/kg in liver of cattle, horses and pigs and 0.3 µg/L in cattle milk based on an ADI of 0–0.015 µg/kg of body weight. Considerable metabolism of dexamethasone was noted. However, the metabolites do not have any biological activity and, consequently dexamethasone, was proposed as a marker residue. MRLs were designated as temporary because there was no adequate method to determine compliance with the MRL.

Performance data was requested on the dexamethasone analytical method for evaluation at the 48th meeting of the Committee but no data were received for evaluation. The Committee decided to withdraw the temporary MRLs for dexamethasone due to lack of an adequate analytical method allowing enforcement of the MRLs. At the present meeting the Committee reviewed documentation for the HPLC-MS method for control of dexamethasone residues in tissues and milk.

General

Liquid chromatographic methods based on UV detection were considered unsuitable for residue analysis at sub-µg/kg concentrations. Although a method for analysis of dexamethasone in samples at 0.1 µg/L by gas chromatography/mass spectrometry using negative chemical ion monitoring has been described, attempts to apply this method for food commodities failed. Immunoassays were considered to meet the required detection levels but other technical problems were encountered. A liquid chromatography thermospray mass spectrometry (TS-LS/MS) method was developed. The essential studies were performed in accordance to GLP. The appropriate references and statements were provided.

TS-LC/MS based methods require high quality laboratories to maintain the complex and expensive equipment and skilled operating personnel. Failure to maintain instrument performance may adversely affect method reproducibility. The transferability of such a method is questionable and this limits its use as a regulatory method.

Analytical Method

Tissue and milk sample preparation was performed using liquid/liquid extraction. The sample is homogenized in buffer, extracted and purified and transferred to the chromatographic system.

The chromatographic method involves gradient elution using a reversed phase column. The chromatograms provided with the report showed some apparent retention time instabilities. The report did not offer any explanation for this phenomenon.

Large variation in detector response was reported to occur during analysis. Non-specific interferences are encountered occasionally, requiring adjustments to the concentrations found. While this can be done in fortified samples, such correction can not be done accurately in incurred residue samples. Therefore, calculation of quantitative results in incurred samples may not be accurate.

Prednisolone, cortisone, methylprednisolone, triamcinolone, flumethasone, and isoflupredone did not interfere with the dexamethasone analysis. However, betamethasone, an isomer of dexamethasone, was found to elute together with dexamethasone. Consequently, the method does not make an unambiguous identification of dexamethasone .

Method validation

Linearity was considered acceptable when the correlation coefficient exceeded 0.98. The recovery and accuracy of the method were determined by fortifying 5 replicates at the LOQ, twice the LOQ, 10 times the LOQ, and by use of blank tissues. The method failed to report absolute recoveries. Precision was calculated as coefficient of variation of the concentrations of the fortified samples. The criteria set by the Codex Alimentarius, Residues of Veterinary Drugs in Foods, Vol 3 for accuracy and precision were used and fulfilled. The limit of detection (LOD) was determined as the concentration that gave a signal to noise ratio greater than 3 and limit of quantification (LOQ) as the lowest concentration at which acceptable precision and accuracy

were recorded. The claimed LOQs were 0.5 μg/kg for muscle, kidney and fat tissues in bovine, porcine and equine species and 0.5, 1.0 and 1.0 μg/kg in the liver tissue of the respective species. The LOQ for porcine skin was 0.5 μg/kg and for bovine milk 0.25 μg/kg.

Conclusion

The method did not meet the required performance criteria for identification and quantification of incurred residues in tissues. Therefore, the method was not considered to be suitable for regulatory dexamethasone residue analysis. The Committee agreed that the MRL showed remain withdrawn in absence of an acceptable analytical method for regulatory purposes.

REFERENCES

Wells, R. (1994a). Dexamethasone in JECFA Monograph "Residues of some veterinary drugs in animals . and foods". FAO Food and Nutrition Paper 41/6 pp. 13-31.

Wells, R. (1994b). Dexamethasone in JECFA Monograph "Residues of some veterinary drugs in animals and foods". FAO Food and Nutrition Paper 41/7 pp. 15-16.

Cook, J. and McCormack, A. (1996). Determination of specificity of a dexamethasone assay with respect to other corticosteroids. Final Report (1043/14-1012). Corning Hazleton Europe, North Yorkshire, England.

Curl, M.G. and McCormack, A. (1996). Development and validation of an analytical method for the determination of dexamethasone in tissues and plasma of cattle, pigs and horses and in milk of cattle. Amended Final Report (7309-806/3). Corning Hazleton Europe, North Yorkshire, England

DICLAZURIL

First draft prepared by
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Federal Institute for Health Protection of
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ADDENDUM

to the Diclazuril monograph prepared by
the forty-fifth meeting of the Committee and published in
FAO Food and Nutrition Paper 41/8, Rome 1996

At its forty-fifth meeting the Committee had recommended temporary MRLs for residues of diclazuril in certain tissues of food animals expressed as parent compound. The MRL's were temporary because the ADI was temporary.

No additional residue data were provided to the Committee for consideration at its current meeting. Since the final ADI established by the Committee at its current meeting was not lower than the temporary ADI allocated at the 45th meeting and since the temporary MRLs already reflected good practice in the use of this veterinary drug the Committee decided to delete the temporary qualification and recommend MRLs at the same level for the same species/tissue combinations as had been recommended at its forty-fifth session.

The recommended MRLs expressed as parent compound, are:

Species	Animal Tissue	MRL (µg/kg)
Sheep, rabbits	Liver	3000
	Kidney	2000
	Fat	1000
	Muscle	500
Poultry	Liver	3000
	Kidney	2000
	Skin/Fat	1000
	Muscle	500

EPRINOMECTIN

First draft prepared by
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Australian Government Analytical Laboratories
Pymble, Australia

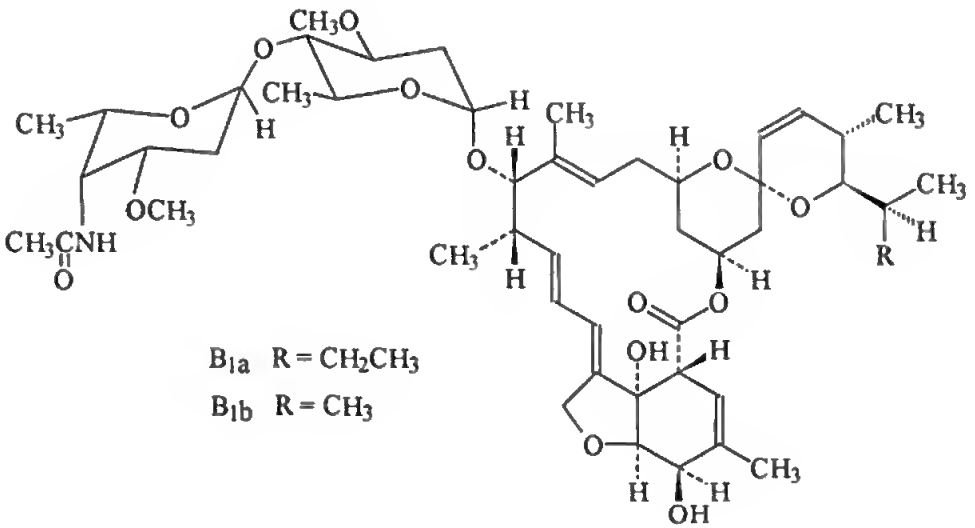
IDENTITY

Chemical name:

Eprinomectin B_{1a}:- (2a*E*,4*E*,5'*S*,6*S*,7*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*, 20a*R*, 20b*S*)-6'-[(*S*)-*sec*-butyl]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethyl-17-oxospiro-[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclo-octadecin-13,2'-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl-a-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-a-L-*arabino*-hexopyranoside (IUPAC).
(4"R)-4"-(acetylamino)-5-O-demethyl-4"-deoxyavermectin A_{1a} (CAS)

Eprinomectin B_{1b}:- (2a*E*,4*E*,5'*S*,6*S*,6'*R*,7*S*,8*E*,11*R*,13*S*,15*S*.17a*R*,20R,20a*R*, 20b*S*) - 5',6,6',7,10,11,14, 15,17a,20,20a,20-dodecahydro-20,20b-dihydroxy-6'-isopropyl-5',6,8,19-tetramethyl-17-oxospiro-[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*]-[2,6]benzodioxacyclo-octadecin-13,2'-[2*H*]pyran]-7-yl-4-*O*-(4-acetamido-2,4,6-trideoxy-3-*O*-methyl-a-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-3-*O*-methyl-a-L-*arabino*-hexo-pyranoside (IUPAC).
(4"R)-4"-(acetylamino)-5-O-demethyl-25-de-(1-methyl-propyl)-4"-deoxy-25-(1-methylethyl)avermectin A_{1a} (CAS)

Chemical structure:



B_{1a} R = CH_2CH_3
B_{1b} R = CH_3

Molecular formula:

$C_{50}H_{75}NO_{14}$ (eprinomectin B_{1a})
$C_{49}H_{73}NO_{14}$ (eprinomectin B_{1b})

Molecular weight: 914.14 (eprinomectin B_{1a})
900.11 (eprinomectin B_{1b})

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Purity: Eprinomectin consists a mixture of two closely related compounds which are semisynthetic analogues of the avermectin group of natural products. Eprinomectin is defined as comprising of a mixture of eprinomectin B_{1a} and eprinomectin B_{1b} in which eprinomectin B_{1a} constitutes no less than 90% and eprinomectin B_{1b} constitutes no more than 10% of the mixture and in which eprinomectin B_{1b} plus eprinomectin B_{1a} constitute a minimum of 95% of the eprinomectin content of the drug. The drug is stabilised by addition of up to 1.2% vitamin E as an antioxidant and the end use pour-on product is further stabilised with butylated hydroxytoluene.

Appearance: White crystalline solid

Melting point: 173°C (dec.)

Optical rotation: $[\alpha]_{405nm}^{25°}$ = 125°-135° (c = 0.5, chloroform)

Solubility (g/L):

water	0.0035±0.002
propylene glycol	>400
propylene glycol octanoate decanoate	199
oleyl alcohol	180
acetylated lanolin	38.4
isostearyl stearate	17.4
cetearyl octanoate	9.4

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Eprinomectin is a semi-synthetic mixture derived from abamectin by replacement of the equatorial hydroxyl group on C4" with an axial acetamino substituent. The two substances that comprise eprinomectin differ from each other only by one methylene group in a side chain substituent. Thus, a secondary butyl side chain in eprinomectin B_{1a} occurs as an isopropyl side chain in eprinomectin B_{1b}. Eprinomectin is more hydrophilic than either abamectin or ivermectin while retaining the potent parasitic properties of the avermectins.

Dosage

Eprinomectin is supplied as a topical pour-on formulation (0.5% in propylene glycol octanoate decanoate) applied along the mid-line of the animal's back. It is used as an endo- and ectoparasiticide for both beef and lactating dairy cattle at a recommended dose of 0.5 mg/kg.

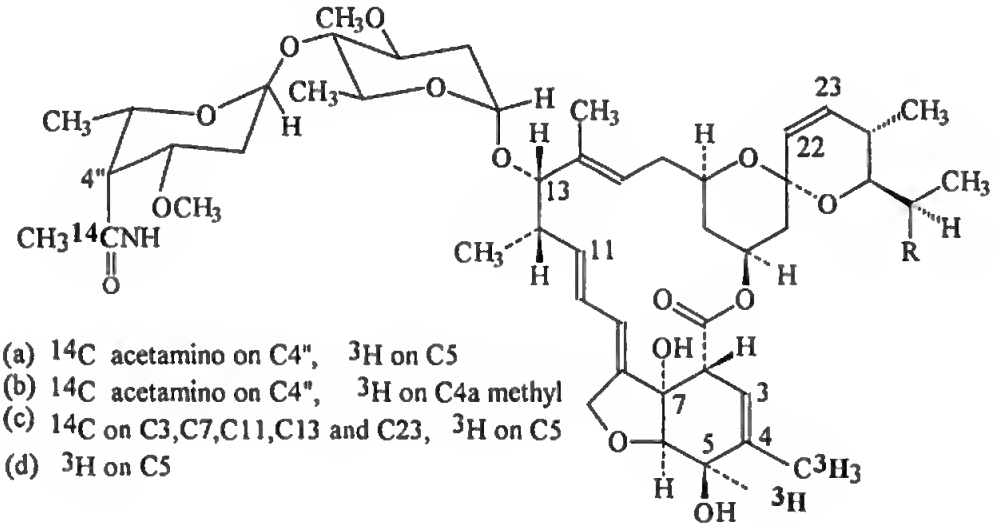
METABOLISM

Radiolabelled Eprinomectin

Various excretion and pharmacokinetic studies were conducted with 3 separate preparations of eprinomectin double labeled with ^{14}C and ^{3}H together with one analogue, singly labeled with ^{3}H. The molecular labeling sites for these preparations are shown in Figure 1.

One very important aspect of using different double radio-labeled preparations in pharmacokinetic studies was to determine if the ^{3}H radiolabel on C5 was labile. It was found, in a number of separate studies, that the ratio of radioactivity of ^{14}C at various positions to that of ^{3}H on C5 was constant and that the loss of ^{3}H from C5 was less than 2% in any study. Therefore the stability of the ^{3}H radio-label on C5 has been established, enabling the use of less costly singly labeled [5-^{3}H] eprinomectin [Figure 1(d)] in subsequent radio-labeled depletion studies.

Figure 1 **Structures of ^{3}H and ^{14}C Doubly Radio-labeled Preparations of Eprinomeetin Used in Pharmacokinetie, Metabolism and Residue Depletion Studies**



Pharmacokinetics

Bioavailability and Proteln Binding

Four Holstein dairy cows (523 - 666 kg BW) were administered approximately 25, 50 and 100 µg/kg BW eprinomectin IV as infusions every four hours followed by a final topical dose of 500 µg/kg BW along the backs of the animals (Faidley *et al.*, 1995). Plasma eprinomectin concentrations, measured by HPLC, showed that after the topical application, peak plasma values were reached in 2 - 5 days and the drug could be detected for up to 21 days with a mean residence time of 165 h (range: 132 - 200h). Bioavailability was 0.29 (range: 0.21 –0.36). Most absorption occurred within 7 - 10 days following an initial lag time of 24 h. However, absorption was still occurring to a minor extent between 17 - 21 days. An in vitro study demonstrated that eprinomectin is >99% bound to bovine plasma proteins at concentrations typical of those found in *in vivo* studies (Venkataraman, 1995)

Exeretion and Metabolism

Rats

Six male Sprague-Dawley rats were each orally dosed with approximately 15.5 mg/kg BW eprinomectin B_{1a}, doubly radio-labelled with ^{14}C and ^{3}H as shown in Figure 1c (Narasimhan and Venkataraman, 1993). Three rats were sacrificed at both 24 h and 48 h after dosing. Total radioactive residues in all tissue and excreta were determined. Less than 0.5% of the dose was excreted in the urine, with nearly all (76-99%) of the administered dose appearing in the faeces during 0-48 h. Very little loss of ^{3}H as water was detected and the ^{14}C:^{3}H ratio remained constant and nearly equal to 1.0 in both tissues and faeces.

Both the parent drug and its metabolites could be quantitatively extracted from liver and faeces with organic solvents. Eprinomectin B_{1a} was the major residue in both liver and faeces, accounting for 82% of the total radioactivity after 24 h and

73% after 48 h in liver and for 85% from 0-24 h and 80% from 24-48 h of the total radioactive residues in faeces. At least five metabolites (M1-M5) were detected. The metabolite profiles in liver and faeces after 24 and 48 h are shown in Table 1. The major liver metabolite, M5, accounted for about 18% of the total residues at 48 h.

Table 1. **Drug-metabolite profiles in liver and faeces of rats treated orally with doubly radio-labeled ^{14}C and ^{3}H eprinomectin after 24 and 48 h.**

Drug/Metabolite	Percentage of total radioactivity			
	Liver		Faeces	
	24 h	48 h	0-24 h	24-48 h
Eprinomectin B_{1a}	81.98	73.01	84.91	79.96
Metabolite M1	2.79	-	3.45	4.00
Metabolite M2	1.67	-	-	2.05
Metabolite M3		-	3.55	1.64
Metabolite M4	6.46	-		4.52
Metabolite M5	4.54	18.02		1.77

In a related study, 12 male and 12 female Sprague-Dawley rats were each orally dosed with approximately 6 mg/kg BW [5-^{3}H]-eprinomectin B_{1a} for 7 consecutive days (Halley *et al.*, 1995). Three rats of each sex were sacrificed at 7 h, 1 day, 2 days and 5 days after the last dose (Table 2). As in the previous study, the predominant route of excretion was in the faeces with urinary excretion accounting for less than 1%. Parent drug and all radioactive metabolites could be quantitatively extracted from both tissues and faeces with organic solvents. Eprinomectin (eprinomectin B_{1a} plus eprinomectin B_{1b}) was the major residue in all tissues and plasma at 7 hours after the last dose and in faeces at 1 day after the last dose. Metabolite M5 (N-deacetyleprinomectin) was usually the major metabolite in most samples. Comparison of the metabolic profiles of male with female rats at the same time point revealed that less parent drug and more N-deacetyl metabolite was found in females, indicating that the drug is more extensively metabolized in females. Levels of total radioactivity in tissues were in the order: liver ~ fat ~ kidney > muscle > plasma and were similar in both male and female rats.

Eprinomectin has an excretion pathway similar to other avermectins. However, no studies were undertaken to confirm predominant biliary excretion in the rat.

Table 2. **Drug metabolite profiles in liver, kidney and faeces of male rats treated orally with [5-^{3}H] eprinomectin for seven consecutive days**

Drug/Metabolite	Percentage of total radioactivity								
	Liver			Kidney			Faeces		
	7 h off*	1 d off*	2 d off*	7 h off*	1 d off*	2 d off*	1 d on**	1 d off*	4 d off*
Eprinomectin B_{1a}	82.1	76.2	66.7	82.0	72.5	63.0	81.8	80.3	56.8
Eprinomectin B_{1b}	7.7	6.6	2.1	7.3	3.8	1.3	6.9	6.3	2.4
Metabolite M1	1.0	1.0	1.3	1.3	1.1	0.9	2.5	3.0	4.8
Metabolite M2	0.5	-	-	-	-	-	-	1.1	1.8
Metabolite M3	-	-	-	-	-	-	0.6	-	1.6
Metabolite M4	1.5	0.9	1.5	1.7	1.6	0.7	2.6	2.8	2.6
Metabolite M5	4.0	11.6	23.0	5.2	16.4	27.9	0.8	1.2	20.1
Metabolite M5a	-	-	1.1	-	1.4	-	-	-	-

* 'x' days off represents the sampling time after withdrawal of drug.
** 1 day on represents sampling time from commencement of treatment

Cattle

In the first of two radio-labeled studies with dairy cows (Study 1), four lactating dairy cows (468 - 543 kg BW) were dosed topically with 750 µg/kg BW of doubly labeled ^{14}C-^{3}H-eprinomectin and sacrificed 21 days after dosing (Green-Erwin *et al.*, 1994a). Two cows received doubly labeled eprinomectin shown in Figure 1a whereas the other two received an equal dose of the doubly labeled material shown in Figure 1b. In each case, only eprinomectin B_{1a} was labeled. Maximum total radioactivity levels in the faeces of two of the animals were in the range 820 - 2988 µg/kg. The average amount of the drug dose excreted in the faeces was estimated to be 31.8% after 14 days and 34.2% after 21 days (Narisimham, 1995a). Total radioactivity of the parent drug and its metabolites was almost quantitatively solvent extracted from liver, faeces and milk. The metabolite profiles, determined in these three matrices, are shown in Table 3 (Study 1). They demonstrate that eprinomectin is only metabolized to a small extent in milk and liver. The parent drug is the predominant residue and is therefore the appropriate marker residue. The loss of tritium from the ^{3}H-labeled drug was found to be <1% and no un-tritiated metabolites could be detected in any tissue, therefore a ^{3}H-label at the 5-position of eprinomectin appears to be metabolically stable.

In a second study (Study 2), four lactating dairy cows (535-564 kg BW) were dosed topically with 750 µg/kg BW [5-^{3}H]-eprinomectin (similar B_{1a} + B_{1b} ratio as that present in the commercially available drug) and sacrificed 21 days post dosing (Green-Erwin *et al.*, 1994b). The stability of the tritium label was evidenced by the loss of 0.6% as tritiated water during the time course of the study. Only 0.32% of the radioactivity of the initially applied dose (estimated at approximately 1% of the bioavailable dose from other evidence *[vide infra]*) was excreted into milk during the first 14 days after dosing. The metabolism of the drug and relative drug - metabolite ratios were in line with the results found in Study 1 and are summarized in Table 3.

In both these studies, there was a close correlation between the concentration of total radioactive residues and that of eprinomectin B_{1a}. Residue levels in plasma were about 5 times those in milk and residue depletion proceeded at about the same rate from both fluids. However, peak residue levels and residue depletion curves from plasma and milk were markedly different between animals with highest residue levels occurring anywhere between 1 and 7 days. Indeed, significant animal to animal variation in drug absorption and depletion has been noticed in most studies reported for eprinomectin.

Table 3. **Drug metabolite profiles in liver, milk and faeces of dairy cows given a single topical treatment of 750 µg/kg BW radio-labeled eprinomectin.**

Drug/Metabolite	Percentage of total radioactivity						
	Doubly ^{14}C- and ^{3}H-labelled B_{1a} (^{14}C) (Study1)				^{3}H-labelled B_{1a} + B_{1b} (Study 2)		
	Liver	Milk		Faeces	Liver	Milk	
	Day 21	Day 3	Day 8	Day 8	Day 21	Day 3	Day 8
Eprinomectin B_{1a}	93.8	95.5	94.2	88.7	87.4	85.7	85.5
Eprinomectin B_{1b}					8.4	8.3	7.6
Metabolite M1		1.0	1.4	2.7	0.3	1.3	1.1
Metabolite M2				0.9		0.3	0.3
Metabolite M3	-			1.1	0.3		
Metabolite M4	1.1	0.7	0.7	0.8	1.0	0.5	0.7
Metabolite M4a	1.1			1.7			
Metabolite M5	0.9	1.2	2.7	0.3	0.3	1.0	1.9

Twelve cattle of less than one year of age (6 steers and 6 heifers, 274-336 kg BW) were given a single topical treatment of 500 µg/kg BW of [5-^{3}H] labelled eprinomectin ('natural' mixture of B_{1a} + B_{1b}; Figure 1d), applied along the mid-line of the back (Green-Erwin *et al.*, 1994c). Three animals were sacrificed at 7, 14, 21 and 28 days, respectively, after dosing and urine and faeces collected from two steers from the group that was sacrificed after 28 days. Maximum radioactivity and maximum eprinomectin B_{1a} levels in plasma were in the ranges 4.35 - 21.10 µg/L and 7.33 - 19.74 µg/L, respectively, and were attained 2 - 5 days after dosing. Faecal excretion was the major elimination pathway with 14.35% of radioactivity being excreted in faeces compared to 0.35% in urine during 28 days post dosing. Analysis of the hide of the cattle

sacrificed at 28 days showed that 54% of the applied radioactive dose, of which 89% was undegraded eprinomectin, remained unabsorbed. This evidence suggests that over 30% of the bioavailable dose is excreted in faeces within the first 28 days post dose.

Total radioactive residues could be almost quantitatively solvent extracted from tissues, plasma and faeces. The metabolite profiles were determined for all matrices at different time points (Venkataraman and Narasimhan, 1995). Table 4 shows the drug - metabolite profiles for all matrices averaged over a number of collection time points. The predominant residue in all matrices was the parent drug. This was accompanied by minor quantities of 5–7 metabolites in most matrices, most at levels of about 1% or less. The exception was the occurrence of metabolite M5 in muscle at 3.9% and metabolite M1 in faeces at 7.4%.

It can be concluded that eprinomectin is not metabolised to any great extent in cattle tissues following topical application and that it is excreted, predominantly unchanged, through the faeces.

Table 4. Drug metabolite profiles in liver, kidney, muscle, fat and faeces of cattle given a single topical treatment of 500 µg/kg BW [5-^{3}H] radio-labeled eprinomectin.

Drug/Metabolite	Percentage of total radioactivity					
	Liver	Kidney	Muscle	Fat	Plasma	Faeces
Eprinomectin B_{1a}	86.4	86.2	82.0	86.7	87.4	78.3
Eprinomectin B_{1b}	9.3	9.2	8.9	7.2	7.4	8.3
Total eprinomectin*	94.8	94.5	89.9	93.9	94.8	85.9
Metabolite M1	0.7	1.0	1.0	0.3	0.4	7.4
Metabolite M2	0.3	0.1	0.3	0.2	0.4	1.6
Metabolite M2a				0.3	0.2	
Metabolite M3	0.5	0.2	0.4	0.4	0.4	0.5
Metabolite M3a				0.9	0.2	
Metabolite M4	1.1	1.0	1.2	0.7	0.9	0.9
Metabolite M5	0.6	1.3	3.9	1.0	0.9	0.6

* Sum of eprinomectin B_{1a} and eprinomectin B_{1b}

Metabolites of Eprinomectin

Four of the various metabolites distinguished in various studies detailed above have been identified, as detailed below. In all studies, data supported the expectation that eprinomectin B_{1a} and eprinomectin B_{1b} metabolise at the same rate.

Metabolite Identification Code	Identity
M1	24-demethyl-24-hydroxymethyleprinomectin B_{1a}
M2	24-hydroxyeprinomectin B_{1a}
M3	26-hydroxymethyleprinomectin B_{1a}
M5	N-de-acetyleprinomectin B_{1a}

TISSUE RESIDUE DEPLETION STUDIES

Radio-labeled Residue Depletion Studies

Cattle

Twelve cattle of less than one year of age (6 steers and 6 heifers, 274-336 kg BW) were given a single topical treatment of [5-^{3}H] labelled eprinomectin (Figure 1d) along the mid-line of the back at a dose of 500 µg/kg BW (Green-Erwin *et al.*,

1994c). Three animals each were sacrificed at 7, 14, 21 and 28 days after dosing and total residue and eprinomectin B_{1a} concentrations measured in liver, kidney and fat as well as in two muscle samples, one obtained adjacent to, and the other from a region remote from the dosing site. Results are summarised in Table 5. The half-life for the depletion of total residues was about 8 days in all tissues and total residues, determined by radioactivity, and eprinomectin B_{1a}, measured by HPLC, depleted at a similar rate in all tissues. The ratio of eprinomectin B_{1a} to total residues (with standard deviations in parentheses), averaged over all four time points shown in Table 5 was 0.83(±0.12), 0.85(±0.08), 0.92(±0.09), 0.69(±0.14) and 0.71(±0.15) in liver, kidney, fat, muscle and dose site muscle, respectively.

Table 5. **Total residue and eprinomectin B_{1a} concentrations in cattle given a single topical treatment of 500 µg/kg BW of ^{3}H-radiolabelled eprinomectin (3 animals per time point).**

Tissue		Withdrawal time (days)							
		7		14		21		28	
		Concentration of residues* (total in µg equiv/kg, eprinomectin B_{1a} in µg/kg)							
		Total	B_{1a}	Total	B_{1a}	Total	B_{1a}	Total	B_{1a}
Liver	(mean)	977	807	751	546	465	369	185	181
	(range)	824-1086	625-955	479-931	349-717	202-666	179-567	124-231	102-232
Kidney	(mean)	181	161	121	113	70	54	30	24
	(range)	127-248	114-221	76-146	73-139	38-97	28-71	19-39	16-28
Muscle	(mean)	8	6.3	6	3.5	4	2.7	2	1
	(range)	5-11	3-8	5-7	3-4	2-5	<2-4	1-2	all <2
Muscle	(mean)	24	17	10	7.8	19	14	22	12
(dose site)	(range)	19-29	14-21	6-13	4-11	12-28	7-19	6-52	4-29
Fat	(mean)	34	30	22	19	14	14	5	4.7
	(range)	21-50	22-43	15-26	12-22	6-21	5-19	3-7	3-7

*Mean results derived from 3 animals per time point <LOQ = below limit of quantification in all 3 animals

In two separate studies, lactating cows were treated with 1.5 times the recommended dose of labeled eprinomectin. In each study, total residues were estimated radiometrically and the concentration of eprinomectin B_{1a} was determined by HPLC.

Four lactating dairy cows (468-543 kg BW) were dosed topically with 750 µg/kg BW of doubly labeled ^{14}C and ^{3}H eprinomectin and sacrificed 21 days post dosing (Green-Erwin *et al.*, 1994a). Total residue and eprinomectin B_{1a} concentrations were measured radiometrically, or by HPLC respectively. Maximum total radioactive residue levels in milk occurred within 7 days of dosing, but not at the same time for each animal, and were in the range 8.33 -25.84 µg/L. The concentration of total radioactive residues and of eprinomectin B_{1a} in milk at various times post dosing is shown in Table 6. In general, radioactive residues and eprinomectin B_{1a} concentrations paralleled each other. At 21 days the average total radioactive residue concentrations in liver, dose site muscle, kidney, fat and muscle not adjacent to the dose site were 119.3, 28.6, 15.6, 8.6 and 1.1 µg/kg respectively. The residue levels in two of the four cows were consistently higher than the other two and the elimination of drug in the milk of one cow was much faster and peaked at a much higher value than the other three.

Four lactating dairy cows (535-564 kg BW) were dosed topically with 750 µg/kg BW [5-^{3}H] eprinomectin and sacrificed 21 days post dosing (Green-Erwin *et al.*, 1994b). Total residue and eprinomectin B_{1a} concentrations were measured radiometrically or by HPLC, respectively. Only 0.32% of the radioactive dose was excreted into milk up to 14 days post dose. Maximum total radioactive residue levels occurred within 7 days of dosing and were in the range 3.08-9.02 µg/L. At 21 days the average total radioactive residue concentrations in liver, dose site muscle, kidney, fat and muscle not adjacent to the dose site were 145.8, 87.5, 21.4, 12.2 and 0.7 µg/kg, respectively. In both studies, average residue levels in milk peaked between 2 and 4 days post dosing.

The values of total radioactive residues shown in Table 6 have been corrected for the presence, in the milk, of small amounts of tritiated water arising from the slight loss of tritium label (Narasimhan, 1995b). The ratio of marker residue to total radioactive residues was calculated at each time point in these studies and the average value over nearly 190 samples measured was calculated to be 0.77 ± 0.1.

Six pregnant Holstein dairy cows (537 - 756 kg BW) were topically dosed with 750 µg/kg BW [5-^{3}H] radio-labeled eprinomectin 21 days prior to anticipated delivery date (Green-Erwin *et al.*, 1995). The 8 resulting calves were sacrificed 12 –24 h after birth. Residue levels in the edible tissues were at, or near, assay detection limits in all tissues except liver where total residues averaged 21.4 µg/kg (range: 5.8 - 55 µg/kg). The nearly identical ratio of eprinomectin B_{1a} found in the dosing solution and in calf liver suggested minimal metabolism of parent drug occurred in calf livers. The highest residue levels in milk/colostrum were in the range: 7.1 - 13.2 µg/L between 7 and 15 days post dose, in line with values found in previous studies on lactating cows.

Table 6. **Eprinomectin B_{1a} concentrations in the milk of 8 cows administed a single topical treatment of 750 µg/kg BW of [5-^{3}H] radio-labeled eprinomectin in two separate studies (CA-365 and CA-367, 4 Cows per Study). Total radioactive residue values shown have been corrected for loss of tritium label.**

Days post dosing	Study CA-365				Study CA-367			
	Total radioactive residues (µg/L)*		Eprinomectin B_{1a} concentration (µg/L)#		Total radioactive residues (µg/L)*		Eprinomectin B_{1a} concentration (µg/L)#	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1	6.41	16.42-1.91	5.18	14.20-1.20	1.77	2.38-1.46	1.56	2.19-1.05
2	9.68	15.73-4.54	7.40	12.9-3.20	4.26	6.37-2.20	3.77	5.76-1.78
3	9.15	10.71-5.17	6.78	8.10-3.7-	4.90	8.18-2.53	3.96	6.84-2.04
4	6.61	8.68-5.59	5.10	6.00-4.30	4.03	6.57-2.32	3.12	5.02-1.80
5	5.04	6.42-3.50	3.65	4.60-2.40	3.34	4.75-2.38	2.89	3.97-1.94
6	3.61	4.54-2.51	2.43	3.20-1.30	3.28	5.17-1.97	2.94	4.60-1.65
8	2.70	4.24-1.01	1.58	2.40-0.50	2.87	3.61-1.84	2.50	3.27-1.48
10	2.03	2.82-0.68	1.25	2.00-0.30	2.52	3.21-1.79	2.04	2.68-1.19
12	1.24	1.74-0.25	0.70	<LOD-1.0	1.69	2.07-1.30	1.30	1.16-0.94
t4	t.31	2.07-0.39	<LOD	<LOD	1.50	1.95-0.95	t.09	t.40-0.65

* corrected for loss of tritium as tritiated water

\# measured by a validated HPLC procedure with fluorescence detection

Residue Depletion Studies Using Unlabeled Eprinomectin

Cattle

In an initial residue depletion study (Study 1), 30 Hereford x Holstein beef cattle (15 steers and 15 heifers; 436 - 645 kg BW) were dosed topically, along the mid-line of the back, with a 0.5% solution of eprinomectin in Miglyol 840 at the rate of 500 µg/kg BW (Payne *et al.*, 1995). Groups of 5 animals were slaughtered at 10, 17, 24, 34, 44 and 55 days after application of the dose. Four untreated cattle were necropsied to supply control samples. Residue levels of eprinomectin B_{1a}, the proposed marker residue for eprinomectin, were measured by a validated HPLC method. The results of this study, showing both the concentration range and arithmetic mean of concentration of eprinomectin B_{1a} in each tissue at each time point are shown in Table 7. Recoveries for all tissues were in the range 72 - 111% and each tissue sample was analysed in duplicate, except for 10 day liver samples where 4 separate determinations were conducted. Coefficient of variation of same set replicates and/or repeat tissue analyses were 9%, 3%, 4.8%, 4.6% and 1.4% for liver, kidney, fat, muscle and dose-adjacent muscle, respectively, which demonstrated the reproducibility of the method. Method recoveries for all tissues over

all fortification levels averaged 92% and the proposed marker residue, eprinomectin B_{1a}, comprised about 92% of the B_{1a} + B_{1b} mixture of eprinomectin homologues which, in combination, constitute the commercial drug.

In a second study (Study 2), eprinomectin tissue residue levels were investigated at earlier slaughter times (Payne *et al*,. 1996a). Twenty five Angus or Hereford beef cattle (13 steers and 12 heifers; 227 - 389 kg BW) were dosed topically along the mid-line of the back with IVOMEC®EPRINEX™ Pour-On for Beef and Dairy Cattle (a 0.5% solution of eprinomectin in Miglyol 840) at the rate of 500 µg/kg BW. Two untreated cattle were necropsied to supply control samples. Groups of 5 animals were slaughtered at 0.5 (8-12 h), 1, 3, 5, and 7 days after application of the dose. Residue levels of eprinomectin B_{1a}, the proposed marker residue for eprinomectin, showing both the concentration range and arithmetic mean of concentration of eprinomectin B_{1a} in each tissue at each time point are shown in Table 8.

Table 7. **Eprinomectin B_{1a} concentrations (µg/kg) in the tissues of beef cattle administered a single topical dose of 500 µg/kg BW of eprinomectin. Five animals sacrificed at each time point**

Days post dosing	Liver		Kidney		Muscle		Dose Site Muscle		Fat	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
10	748	637-827	74	57-91	6	4.5-8.2	8	6.2-12	26	9.6-39
17	237	50-360	40	17-56	2	<2-3.2	3	<2-5.9	8	4.1-15
24	56	23-93	9	3-15	<2	<2-<1	<2	<1-2.6	3	<1-7.7
34	26	9-67	4	<2-9.6	NM	NM	<1	<1	<2	<2-<1
44	4	<2-8.2	<1	<1	NM	NM	<1	<1	<1	<1
55	<1	<1	<1	<1	NM	NM	NM	NM	NM	NM

NM = not measured; <2 = below level of quantification; <1 = below level of detection
All values uncorrected for recovery

Table 8. **Eprinomectin B_{1a} concentrations (µg/kg) in the tissues of beef cattle given a single topical treatment of 500 µg/kg BW of eprinomectin. Five animals sacrificed at each time point**

Days post dosing	Liver		Kidney		Muscle		Dose Site Muscle		Fat	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
0.5	278	192-354	34	30-40	2	<2-2	<2	<2-2	6	4-8
1	551	315-703	75	64-93	4	2-6	3	2-4	15	12-18
3	710	471-934	93	66-114	5	3-8	4	3-6	20	11-26
5	376	253-555	55	35-81	3	<2-4	2	<2-3	8	5-12
7	323	247-422	23	19-33	<2	<1-<2	<2	<1-<2	4	3-5

<2 = below level of quantification; <1 = below level of detection; All values uncorrected for recovery

The marker residue, eprinomectin B_{1a}, peaked approximately 3 days after dosing in all tissues assayed. A marker residue range of 471 to 934 µg/kg was found in liver at this time point which decreased to a range of 240 – 430 µg/kg in liver by day 7 post dosing. Depletion from other tissues followed the same pattern as that found for liver. The highest marker residue concentrations found in kidney and fat were 114 µg/kg and 26 µg/kg, respectively.

A comparison between the results reported in Tables 7 and 8 is warranted. The dosing regimen reported for both studies appears identical and both studies were carried out under field use conditions, albeit on different continents, under different climatic conditions and using different breeds of cattle. The HPLC method used to determine residues was the same in

both cases apart from two minor modifications to the analyte extraction conditions used to gather some of the data presented in Table 8. Recoveries from both studies were very similar and exceeded 80% for all tissues. However, in Study 1 (Payne *et al.*, 1995), the mean marker residue concentration in liver after 10 days was 748 µg/kg whereas, in Study 2 (Payne *et al.*, 1996a), the mean marker residue concentration in liver after 7 days 323 µg/kg. Furthermore, in Study 2, the mean Day 7 residue value in liver was less than half the maximum mean values found in liver on Day 3. Comparison of mean Day 10 values reported in Study 1 with those mean Day 7 values reported in Study 2 for kidney, muscle, dose site muscle and fat are 74:23, 6: <2, 8: <1 and 26:4, respectively. In fact, marker residue concentrations for Day 7 in Study 2 (Table 8) are similar to the residue concentrations found at Day 17 in Study 1. The data presented in Study 1 are more in accord with the radioactive depletion data presented in Table 5 than are the data from Study 2. It is interesting to speculate on which day maximal marker residue levels would have occurred in Study 1.

A residue depletion study was conducted in nonruminating beef calves under field use conditions (Payne *et al.*, 1996b). Twelve male Holstein calves, less than 16 weeks old and weighing about 100 kg BW, were treated topically along the back with IVOMEC®EPRINEX™ Pour-On for Beef and Dairy Cattle (a 0.5% solution of eprinomectin in Miglyol 840) at the rate of 500 µg/kg BW. Two untreated calves were necropsied to supply control samples but revealed small amounts of the marker residue. Therefore, eprinomectin-free tissue from a previous study in ruminating animals was used as control samples. Groups of 3 animals were slaughtered at 1, 3, 7 and 14 days after application of the dose. Residue levels of eprinomectin B_{1a} showing both the concentration range and arithmetic mean of concentration of eprinomectin B_{1a} in each tissue at each time point are shown in Table 9.

The highest marker residue concentrations occurred 7 days after treatment in all tissues and the highest residue concentrations were in the liver (1220 µg/kg), followed by fat (287 µg/kg), kidney (237 µg/kg) and muscle (48 µg/kg). Liver residues declined to a mean value of 803 µg/kg after 14 days, but depletion rates in other tissues over this period were greater in this particular and limited study. Marker residue concentrations found in this study were somewhat higher than those found in other residue depletion studies in ruminating animals.

Table 9. **Eprinomectin B_{1a} concentrations (µg/kg) in the tissues of preruminating dairy calves administered a single topical dose of 500 µg/kg BW eprinomectin. Three animals sacrificed at each time point**

Days post dosing	Liver		Kidney		Muscle		Dose Site Muscle		Fat	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1	618	355-1050	119	54-250	25	8-56	9	3-22	172	51-414
3	832	683-925	166	118-213	28	17-40	26	19-41	168	137-201
7	1220	881-1640	237	223-250	48	44-54	57	49-65	287	260-339
14	803	769-855	120	62-170	22	19-23	23	17-26	103	64-140

In a study designed to investigate the effect of eprinomectin residues on the processing of common dairy products, thirty satisfactorily milk-yielding Fleckvieh dairy cows were divided into three groups (Barth *et al.*, 1995). Two groups were treated with a 0.5% solution of eprinomectin (in Miglyol 840) at the rate of 500 µg/kg BW, while the third group served as a control. Quantifiable amounts of eprinomectin B_{1a} were first detected 23 h after treatment. Residues could be detected for between 6 and 12 days post dosing. The maximum residue concentration in milk reached 11.25 µg/L with a peak occurring after 2-3 days in 80% of the animals while the remainder had maximum residue concentrations in their milk up to 7 days after treatment. Yogurt and cheese produced from treated and untreated animals could not be differentiated in organoleptic trials. The milk residue results were similar to those obtained in the smaller radio-labeled dosing trial reported earlier (Green-Erwin *et al.*, 1994a,b). Mean concentrations, concentration ranges and standard deviations of eprinomectin B_{1a} residues in milk are shown in Table 10.

Table 10. **Eprinomectin B_{1a} concentrations in the milk of 20 dairy cows administered a single topical dose of 500 µg/kg BW of eprinomectin.**

Days post dosing (evening milking)	Eprinomectin B_{1a} concentration (µg/L) Mean	Range	Standard Deviation	No. of animals
0	0			20
1	2.47	0.56-6.62	1.82	20
2	4.90	1.29-11.25	3.09	20
3	4.64	1.55-9.18	2.28	20
4	3.48	1.27-7.88	1.53	20
5	2.70	1.30-5.14	1.01	20
6	2.24	0.86-4.52	0.96	20
7	1.62	0.69-2.66	0.65	20
8	1.15	0.67-2.14	0.48	20
9	0.83	0.36-1.83	0.41	20
10	0.65	0.31-1.21	0.25	11
11	0.52	0.41-1.02	0.16	7
12	0.59	0.45-0.74	0.15	3
13	0.63	0.63		1

METHODS OF ANALYSIS IN BOVINE TISSUES AND IN MILK

The detection of members of the avermectin class of compounds at the low concentrations required for residue determination has posed some analytical challenges. Neither GC nor HPLC with UV detection is suitable. A high performance liquid chromatographic fluorescence method for the determination of eprinomectin in bovine tissue and in milk is based on a method used for the determination of abamectin and ivermectin residues. It is based on the quantitative double dehydration of avermectins, with trifluoroacetic anhydride - 1 methylimidazole, to produce a strongly fluorescing aromatic derivative (de Montigny *et al.*, 1990). The process by which eprinomectin is dehydrated by trifluoroacetic anhydride - 1 methylimidazole is analogous to that illustrated for a structurally related substance shown in Figure 2.

Eprinomectin B_{1a} and B_{1b} are isolated from tissue by solvent extraction followed by sample clean up on a solid phase cartridge. The conversion to fluorescent derivatives is achieved by addition of trifluoroacetic anhydride to a solution of eprinomectin in 30% 1-methylimidazole in acetonitrile immediately prior to injection into an HPLC column. The fluorescent derivatives formed from eprinomectin are not as stable as the structurally analogous derivatives prepared from either abamectin or ivermectin. This necessitates the in-line preparation of the derivative immediately prior to analysis by reverse phase HPLC. Nevertheless, this process is readily automated allowing routine overnight analyses to be conducted.

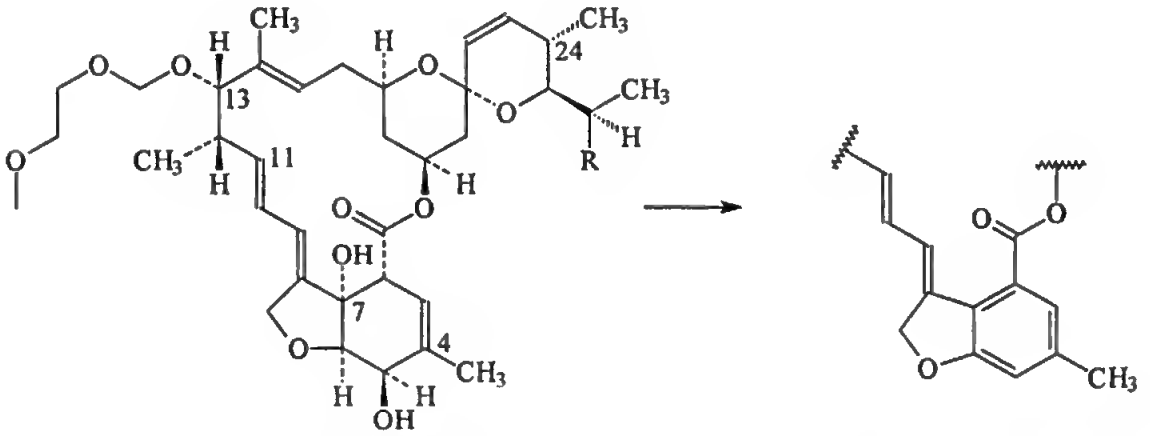
The method, which uses an external standard, was thoroughly validated and can accurately determine eprinomectin residues over a wide concentration range (2-2500 µg/kg). The quantification limit (LOQ) was 2 µg/kg and a limit of detection (LOD) was 1 µg/kg in all tissues and milk. The coefficient of variation for all tissues was 14% or less, with an average of 12% and a range of 11-14%. There were no analytical interferences from abamectin, emamectin, doramectin, moxidectin, ivermectin or coumaphos. The method has proved extremely robust and several different analysts and contract laboratories have successfully used the method. Full disclosure of the method has been recently been accomplished through peer reviewed publication (Payne *et al.*, 1997).

A modified method has been developed for milk in which a 4"-sulfonamide analogue with a close structural relationship to eprinomectin is used as an internal surrogate (de Montigny, 1994). Quantitative extraction recovery was verified with labelled eprinomectin and lack of interference from both abamectin and ivermectin was established. The method was validated between 2 and 50 µg/L and the LOQ and LOD of the method were determined to be 1.0 and 0.25 µg/L

respectively. This method has now been modified by changing the internal standard to a more readily available substance. The structure of the internal standard and of the fluorophore formed by dehydration is shown in Figure 2 (de Montigny and Ocasio, 1994).

The stability of eprinomectin in frozen milk samples for at least 36 days has been established (de Montigny, 1994). Eprinomectin proved stable in frozen samples of all four edible bovine tissue for at least 24 months (Payne *et al.*, 1996c).

Figure 2



surrogate fluorescent derivative

APPRAISAL

Eprinomectin, a semisynthetic analogue of the avermectin group of natural products, is a mixture of two closely related compounds, differing from each other only by one methylene group in a side chain substituent. Eprinomectin B_{1a} constitutes not less than 90% and eprinomectin B_{1b} constitutes not more than 10% of the mixture. Eprinomectin is applied as a topical pour-on formulation. It is used as an endo- and ectoparasiticide on both beef and lactating dairy cattle at a recommended dose of 0.5 mg/kg. Eprinomectin is more hydrophilic than either abamectin or ivermectin while retaining the antiparasitic properties of the avermectins.

Metabolism and Bioavailability

Rats Rats, dosed orally by gavage with [^{14}C-5-^{3}H]-radio-labeled eprinomectin were sacrificed at 24 h and 48 h after dosing. Less than 0.5% of the dose was excreted in the urine, with 76-99% of the administered dose appearing in the faeces during 0-48 h. There was evidence of only minimal loss of the ^{3}H-label as tritiated water. Eprinomectin B_{1a} was the major residue in both liver and faeces, accounting for 82% of the total radioactivity after 24 h and 73% after 48 h in liver and for 85% from 0-24 h and 80% from 24-48 h of the total radioactive residues in faeces. At least five metabolites (M1-M5) were detected. The major liver metabolite, M5 (N-deacetyleprinomectin), accounted for about 18% of the total residues at 48h.

In a related study, rats were each orally dosed by gavage with approximately 6 mg/kg BW [5-^{3}H]-eprinomectin B_{1a} for 7 consecutive days and then sacrificed at 7 h, 1 day, 2 days and 5 days after the last dose. Again, the predominant route of excretion was in the faeces with urinary excretion accounting for less than 1%. Parent drug and all radioactive metabolites could be quantitatively extracted from both tissues and faeces with organic solvents. Eprinomectin was the major residue in all tissues and plasma at 2 days after the last dose and in faeces at 4 days after the last dose. Metabolite M5 was the major metabolite in liver and kidney. Levels of total radioactivity in tissues were in the order: liver ~ fat ~ kidney > muscle > plasma and were similar in both male and female rats.

Cattle In the first of two radio-labeled studies with dairy cows, four lactating dairy cows were treated topically with 750 µg/kg BW [^{14}C-5-^{3}H]-labeled eprinomectin B_{1a} and sacrificed 21 days after dosing. Maximum total radioactivity levels in the faeces of two of the animals ranged from 820 - 2988 µg/kg eprinomectin equivalents. The average amount of the drug dose excreted in the faeces was estimated to be 31.8% after 14 days and 34.2% after 21 days. More than 90% of the total radioactivity of the parent drug and its metabolites was extracted from liver, faeces and milk with ethyl acetate. Eprinomectin was only metabolised to a small extent in milk and in liver. It was the predominant residue and is therefore the appropriate marker residue. In a second study, four lactating dairy cows were dosed topically with 750 µg/kg BW [5-^{3}H]-eprinomectin and sacrificed 21 days post dosing. Only 0.32% of the radioactivity of the initially applied dose (estimated at approximately 1% of the bioavailable dose from other evidence) was excreted into milk during the first 14 days after dosing. In both of these studies, there was a close correlation between the metabolism of the drug and the ratios of concentrations of total radioactive residues to those of eprinomectin B_{1a}. Significant animal to animal variation in drug absorption and depletion was noticed in many studies reported for eprinomectin.

Twelve cattle of less than one year of age were treated with a single topical treatment of 500 µg/kg BW [5-^{3}H] labeled eprinomectin. Three animals per group were sacrificed at 7, 14, 21 and 28 days, respectively, after dosing. Maximum radioactivity as eprinomectin B_{1a} equivalents and eprinomectin B_{1a} levels in plasma were 4.35 - 21.10 µg/L and 7.33 - 19.74 µg/L, respectively, and were attained 2-5 days after dosing. Faecal excretion was the major elimination pathway with 14.35% of radioactivity being excreted in faeces compared to 0.35% in urine during 28 days post dosing. Analysis of the hide of the cattle sacrificed at 28 days showed that 54% of the applied radioactive dose remained in the hide, of which 89% was unmetabolized eprinomectin. More than 90% of the total radioactive residues from tissues, plasma and faeces could be extracted using organic solvent. The predominant residue in all matrices was the parent drug at all time points. This was accompanied by minor quantities of 5–7 metabolites in tissues, plasma and faeces. Most of these metabolites occurred at levels of about 1% or less. The exception was the occurrence of metabolite M5 in muscle at 3.9% and metabolite M1 in faeces at 7.4%. It can be concluded that eprinomectin is not metabolized to any great extent in cattle tissues following topical application and that it is excreted, predominantly unchanged, through the faeces.

Residue Studies in Cattle

Studies in cattle using radiolabeled drug Twelve cattle of less than one year of age were treated with a single topical 500 µg/kg BW dose of [5-^{3}H]-eprinomectin. Groups of three animals were sacrificed at 7, 14, 21 and 28 days after dosing and total residue and eprinomectin B_{1a} residues measured in liver, kidney muscle and fat. The depletion half-life of total residues was about 8 days in all tissues. The ratio of total residues, determined by radioactivity and eprinomectin B_{1a} measured by HPLC depleted at a similar rate in all tissues at all time points. The ratio of eprinomectin B_{1a} to total residues averaged over four time points was 0.83, 0.85, 0.92, 0.69 and 0.71 in liver, kidney, fat, muscle and dose site muscle, respectively. Based on these studies eprinomectin B_{1a} is the marker residue

In two separate studies, lactating cows were treated with 1.5 times the recommended dose using radio-labeled eprinomectin. In each study, total residues were estimated radiometrically and the concentration of eprinomectin B_{1a} was determined by HPLC. In the first of these studies, four lactating dairy cows were dosed topically with 750 µg/kg BW [^{14}C-5-^{3}H]-labeled eprinomectin and sacrificed 21 days post dosing. Maximum total radioactive residue levels in milk occurred within 7 days of dosing, but not at the same time for each animal, and were in the range 8.33 -25.84 µg/L. The mean concentration of total radioactive residues and of eprinomectin B_{1a} in milk peaked two days after dosing. In the second study, four lactating dairy cows were dosed and sacrificed in the same manner as in the first study. Only 0.32% of the radioactive dose was excreted into milk up to 14 days post dose. Maximum total radioactive residue levels occurred within 7 days of dosing and were in the range of 3.08-9.02 µg/L. The concentration of eprinomectin residues in milk peaked three days post dosing. In both studies, the ratio of marker residue to total radioactive residues was calculated at each time point. The average value, based on nearly 190 samples, was calculated to be 0.77 ± 0.10.

Residues in tissues were determined in the two lactating cattle studies described above. At 21 days the average total radioactive residue concentrations in liver, dose site muscle, kidney, fat and muscle not adjacent to the dose site were 119.3, 28.6, 15.6, 8.6 and 1.1 µg/kg respectively. The residue levels were consistently higher in two of the four cows and the elimination of drug in the milk of one cow was much faster and peaked at a much higher value than the other three. At 21 days the average total radioactive residue concentrations in liver, dose site muscle, kidney, fat and muscle not adjacent to the dose site were 145.8, 87.5, 21 4, 12.2 and 0.7 µg/kg, respectively

Six pregnant Holstein dairy cows were treated topically with µg/kg BW [5-^{3}H]-labeled eprinomectin 21 days prior to anticipated delivery date. The 8 resulting calves were sacrificed t2 –24 h after birth. Residue levels in the edible calf

tissues were at or near assay detection limits in all tissues except liver where total residues averaged 21.4 µg/kg (range: 5.8 - 55 µg/kg).

Studies in cattle using unlabeled drug In an initial residue depletion study using unlabeled eprinomectin, 30 beef cattle were treated topically with eprinomectin at 500 µg/kg BW. Groups of five animals were slaughtered at 10, 17, 24, 34, 44 and 55 days after dosing. Residue levels of eprinomectin B_{1a}, were measured by HPLC. Recoveries for all tissues were 72-111% and relative standard deviations of the analytical method were less than 10% in all tissues. At ten days post dosing, mean eprinomectin residues in liver, kidney muscle and fat were 748, 74, 6 and 26 µg/kg, respectively, declining to 56, 9, <2 and 3 µg/kg, respectively, in these same tissues 24 days post dosing.

In a second study with unlabelled eprinomectin, tissue residue levels were investigated at earlier post treatment times. Twenty-five beef cattle were treated topically with 500 µg/kg BW eprinomectin. Groups of five animals were slaughtered at 0.5, 1, 3, 5, and 7 days after application of the dose. Mean eprinomectin values in liver, kidney muscle and fat reached a maximum at three days post-dosing with values of 710, 93, 5 and 20 µg/kg, respectively, declining to 323, 23, <2 and 4 µg/kg, respectively, in these same tissues 7 days post dosing.

A residue depletion study was conducted in 12 non-ruminating male Holstein calves under normal use conditions. The calves, less than 16 weeks old, were treated topically with 500 µg/kg BW eprinomectin. Groups of three animals were sacrificed at 1, 3, 7 and 14 days after dosing. The highest marker residue concentrations occurred 7 days after treatment in all tissues and the highest mean residue concentrations were: in liver, 1220 µg/kg; fat, 287 µg/kg; kidney, 237 µg/kg; and muscle, 48 µg/kg. Liver residues declined to a mean value of 803 µg/kg after t4 days.

In a study designed to investigate the effect of eprinomectin residues on the processing of common dairy products, thirty milk producing dairy cows were divided into three groups. Two groups were treated topically with 500 µg/kg BW eprinomectin, while the third group served as a control. Quantifiable amounts of eprinomectin B_{1a} were first detected 23 h after treatment. Residues could be detected between 6 and 12 days post dosing. The maximum residue concentration in milk reached 11.25 µg/L with a peak occurring after 2-3 days in 80% of the animals while the remainder had maximum residue concentrations in their milk up to 7 days after treatment. Yogurt and cheese produced from treated and untreated animals could not be differentiated in organoleptic trials. The eprinomection residue in milk were similar to those obtained in radiolabeled dosing studies using few animals.

Analytical Methods

A high performance liquid chromatographic fluorescence method for the determination of eprinomectin in bovine tissue and in milk has been developed. It is based on formation of a strongly fluorescing aromatic derivative. The fluorescent derivative of eprinomectin has a half-life of approximately 2 hours and is not as stable as the structurally analogous derivatives prepared from either abamectin or ivermectin. This necessitates the in-line preparation of the derivative immediately prior to analysis by reverse phase HPLC. Nevertheless, this process is readily automated allowing routine overnight analyses. The method, which uses an external standard, was thoroughly validated and can accurately determine eprinomectin residues over a wide concentration range (2-2500 µg/kg). The limit of quantification (LOQ) was 2 µg/kg and the limit of detection (LOD) was 1 µg/kg in all tissues. The coefficient of variation for all tissues was 14% or less, with an average of 12% and a range of 11-14%. There were no analytical interferences from related drugs.

A modified method has been developed for milk using an internal standard that is an analogue of eprinomectin. Quantitative extraction recovery was verified with radiolabeled eprinomectin and the lack of interference from both abamectin and ivermectin was established. The method was validated at 2-50 µg/L and the LOQ and LOD of the method were determined to be 1.0 and 0.25 µg/L respectively.

Considerations in Statistical Approach to MRLs

The committee reviewed all four depletion studies in applying a statistical approach in recommending MRLs for eprinomectin. The committee recognised that the residue depletion data for one study in non-ruminating calves are different from those in three studies in ruminating cattle. In particular, the residues in fat and muscle in the calf study were higher than those found in all three studies involving cattle and required reappraisal of the MRLs. Nevertheless, a set of MRLs can be recommended for all tissues and milk which are consistent with all data provided.

Maximum Residue Limits

Based on the ADI of 0-10 µg/kg for the parent drug established by the Committee, the permitted daily intake of the drug and/or its equivalents is 600 µg for a 60-kg person. In arriving at recommendations for MRLs in various matrices the committee took the following factors into consideration.

- The drug is for use in dairy and beef cattle.
- The limit of quantification of the analytical methods are 1.0 µg/L and 2.0 µg/kg for milk and tissues, respectively.
- The marker residue is always the predominant residue in both tissues and milk.. In milk and muscle, the mean average ratio of eprinomectin B_{1a} to total residues (EPB_{1a}/TR) is 0.69 in muscle, 0.77 in milk, 0.83 in liver, 0.85 in kidney and 0.92 in fat.
- The completeness of the total data set provided by the sponsor allowed MRL values in cows to be derived statistically.
- In recommending the MRLs, the Committee took into account the ratio of total residues in all tissues over the total residue depletion times reported by the sponsor.
- EPB_{1a}/TR factors used to set MRLs for eprinomectin in non-ruminating calves were those established by radiometric studies for cattle. The Committee considered that the metabolism of eprinomectin in calf tissues would probably be less than or equal to metabolism of the drug in the same tissues in cattle.

The committee recommends the following MRL values in bovine tissues and milk expressed as eprinomectin equivalents:

Tissue	Recommended MRL(µg/kg)	Daily Allowance (g)	EPB_{1a}/TR	Total residues (µg)
Muscle	100	300	0.69	44
Liver	2000	100	0.83	241
Kidney	300	50	0.85	18
Fat	250	50	0.92	14
Milk	20 (µg/L)	1500	0.77	39
Total				**356**

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FEBANTEL, FENBENDAZOLE AND OXFENDAZOLE

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ADDENDUM
to the fenbendazole, febantel and oxfendazole residue monographs prepared by the 38th and 45th meetings of the Committee and published in FAO Food and Nutrition Paper 41/4, Rome 1991 and FAO Food and Nutrition Paper 41/8, Rome 1996, respectively.

INTRODUCTION

The Committee has previously considered the three anthelmintic agents febantel, fenbendazole and oxfendazole at the thirty-eighth and forty-fifth meetings. At the thirty-eighth meeting the Committee recommended common MRLs for each of the three drugs using oxfendazole sulfone as the marker residue. Utilizing a temporary ADI of 0 - 4 μg per kg of body weight, the following temporary MRLs, expressed as the sum of the three principle metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) calculated as oxfendazole sulfone equivalents, were recommended for cattle, sheep, and pigs: muscle, fat, and kidney, 100 μg/kg; liver, 500 μg/kg; milk (cow), 100 μg/L.

The Committee requested that the following additional residue information be submitted:

- Studies on the total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone), measured as oxfendazole sulfone, in the edible tissues of cattle and sheep and in the milk of cattle over a 28-day withdrawal period after treatment of animals with fenbendazole or oxfendazole. In particular, information was requested on the use of the pelleted form of fenbendazole in cattle and sheep.

- Studies on the total residues of the above three metabolites, measured as oxfendazole sulfone, in the edible tissues of pigs given fenbendazole and observed over a 7 - 14 day withdrawal period.

- Information on the bioavailability of bound residues in liver after administration of febantel to one of the following species: cattle, pigs, or sheep.

- Development of a suitable method for the determination of total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone, measured as oxfendazole sulfone) in milk.

At the forty-fifth meeting several residue studies following administration of fenbendazole in cattle, sheep and pigs were reviewed. However, the residue-depletion studies on total residues of fenbendazole, oxfendazole and oxfendazole sulfone in cattle and sheep following the administration of febantel and oxfendazole were ongoing. In addition to the results from these studies using febantel and oxfendazole, the Committee noted that, with the increasing production of goats in developing countries, residue data would be required for establishing MRLs in this species.

The results of the depletion studies for febantel and oxfendazole in cattle and sheep as well as three new studies with fenbendazole, one study in the horse and two in pigs, are summarized in this report. Also, information on the pharmacokinetics and residue depletion of fenbendazole and oxfendazole in goats, sheep and cattle are compared. In addition a single method for all three drugs in milk and tissues is evaluated. The method measures the sum of the three principle metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) in both edible tissues and milk as equivalents of oxfendazole sulfone. The limit of quantification (LOQ) of this method in all tissues and milk is claimed by the sponsor to be 5 μg/kg and 5 μg/L, respectively.

METABOLISM AND PHARMACOKINETIC STUDIES

Goats

The *in vitro* oxidative metabolism of fenbendazole (FBZ) has been studied using liver preparations in a number of species including cattle, sheep and goats. All species investigated produced the sulfoxide metabolite (oxfendazole, FBZ-SO) and upon further oxidation, the sulfone (oxfendazole sulfone, FBZ-SO_2) but at varying rates. The rates of metabolite formation in cattle, sheep and goats are given below in Table 1. Although some degree of species specific preference in the rate of formation of known metabolites was seen, the differences were not considered substantial (Short *et al.*, 1988).

Table 1. The rates of metabolite formation, in picomoles/g liver/min, from in vitro studies using liver preparations of cattle, sheep and goats

Species	Total metabolites	FBZ-SO_2	FBZ-SO	FBZ-OH
cattle	454.79	7.26	427.37	20.16
sheep	560.63	18.90	541.74	N.D.
goat	563.87	72.39	387.61	103.33

N.D. = Not detected

The disposition of fenbendazole has been studied in the plasma, urine and feces of goats after oral and IV administration. Fenbendazole, oxfendazole and oxfendazole sulfone were the major drug-related constituents in plasma. Minor amounts of FBZ-OH and FBZ-NH_2 were observed in plasma. The authors concluded that the metabolism of FBZ in the goat is similar to that in other species including cattle and sheep (Short *et al.*, 1987).

The pharmacokinetics of oxfendazole in goats was compared to that in sheep. After intravenous administration of 7.5 mg/kg BW to sheep and goats, the AUCs of oxfendazole were not significantly different. Similarly, the total AUCs for the three metabolites were not significantly different. However, the bioavailability of oxfendazole in goats after oral administration was only about 42% of that in sheep (Bogan *et al.*, 1987).

Pigs

The pharmacokinetics of a 4% powder fenbendazole formulation versus a 1.5% pellet formulation (dose rate 5 mg/kg BW) was determined in pigs (two period crossover bioequivalence study). Comparing the mean pharmacokinetic parameters of all 12 pigs after administration either of the pellets or powder, the maximum concentrations (C_{max}), the times of maximum concentrations (T_{max}) and the AUCs of fenbendazole and oxfendazole were similar (Schmid, 1997).

RESIDUE DEPLETION STUDIES

All values for residue concentrations in tissues in this section, except the study in goats with fenbendazole, were obtained by a method that determines the sum of the three principle metabolites calculated as the oxfendazole sulfone equivalents. The plasma values were determined by a method that measures the three metabolites individually.

Cattle

A single dose of febantel 10% suspension was administered orally to 16 cattle at 7.5 mg/kg BW. At day 7, 14, 21 and 28 days post dose 4 treated animals were sacrificed each time. Two untreated control animals were slaughtered on the day of administration and two more 28 days after dosing. Tissue samples of muscle, fat, liver and kidney were taken from all animals. The results of this study are summarized in Table 2 (Schmidt, 1994a).

Table 2. **Mean tissue concentrations (µg/kg) of oxfendazole sulfone in cattle after a single oral administration of 7.5 mg febantel/kg BW (n=4).**

Days after administration	Muscle	Liver	Kidney	Fat
7	<LOQ	115	LOQ - 6	19
14	<LOQ	7*	<LOQ	LOQ - 8
21	<LOQ	<LOQ	<LOQ	<LOQ
28	<LOQ	<LOQ	<LOQ	<LOQ

*n=3; LOQ = 5 µg/kg

Thirty two animals, sixteen steers and sixteen heifers, were orally administered oxfendazole suspension at a single dose of 4.5 mg/kg BW. Four animals were used as controls. Samples of plasma were collected from treated and control animals at day 1. Samples of fat, kidney, liver and muscle were collected from treated animals slaughtered on days 10, 12, 14, 16, 18, 20, 22 and 24 of the study. Residues of oxfendazole and its metabolites were undetectable in kidney and muscle between 10 and 24 days post treatment. Mean levels of metabolites present in liver had fallen to <20 µg/kg by day 14 declining to <10 µg/kg by day 18. Levels in fat were <10 µg/kg on day 10 declining to undetectable levels by day 14 (Hunt, 1996).

Cattle (milk)

A single dose of febantel 10% suspension was administered orally to 8 lactating cows at 7.5 mg/kg BW. Milk samples were taken from 12 animals (4 of them were untreated) 3 days before and 5 days after administration. Two samples were collected at each day, one in the morning and one in the evening. The results of this study are summarized in Table 3 (Schmidt, 1994b).

Table 3. **Mean concentrations (µg/L) of oxfendazole in milk of 8 lactating cows administered a single oral dose of febantel 10% suspension at 7.5 mg/kg BW.**

Hours after administration	Day of Admin.	Oxfendazole sulfone in the milk(µg/L)	Hours after administration	Day of Admin.	Oxfendazole sulfone in the milk(µg/L)
10	0, Afternoon	172*	82	3, Afternoon	19**
24	1, Morning	256	96	4, Morning	LOQ - 20
34	1, Afternoon	268	106	4, Afternoon	LOQ - 10
58	2, Afternoon	107	120	5, Morning	<LOQ
72	3, Morning	44**	130	5, Afternoon	<LOQ

*n = 6; **n = 7; LOQ = 5 µg/L

Eight lactating cows were given a single oral dose of 9% oxfendazole suspension at a dose of 4.5 mg/kg BW. Individual milk samples were collected from each cow immediately prior to treatment and every 12 hours thereafter up to 120 hours post treatment. Plasma samples were collected at 24 hours post treatment. The mean concentration of residues in plasma were: oxfendazole (268 µg/L); fenbendazole (101 µg/L) and oxfendazole sulfone (268 µg/L). The residue concentration of oxfendazole sulfone in milk are summarized in Table 4 (de Montigny, 1996a).

Table 4. Concentration of oxfendazole sulfone (µg/L) in milk from 8 lactating cows administered a single oral dose of 4.5 mg oxfendazole/kg BW.

Hours after administration	Mean	Range	Hours after administration	Mean	Range
Pre-treatment*	<5	<5	72	19	8 - 32
12	<5 - 87	<5 - 127	84	<5 - 15	<5 - 15
24	222	163 - 261	96	<5 - 7	<5 - 7
36	186	116 - 226	108	<5	<5
48	106	52 - 161	120	<5	<5
60	55	20 - 82			

* immediately prior to drug administration

Sheep

A single dose of febantel 2.5% suspension was administered orally to 16 sheep at 5.0 mg/kg BW. At day 3, 7, 14 and 21 days post dose 4 treated animals were sacrificed each time. The untreated control animals were slaughtered on day of administration and 21 days after dosing, two animals each. The results of this study are summarized in Table 5 (Schmidt, 1995a).

Table 5. Mean tissue concentrations (µg/kg) of oxfendazole sulfone in sheep after a single oral administration of 5.0 febantel/kg BW (n = 4 animals)

Days after administration	Muscle	Liver	Kidney	Fat
3	40	4617	199	133
7	<LOQ	942	11	9
14	<LOQ	123	<LOQ	<LOQ
21	<LOQ	<LOQ-10	<LOQ	<LOQ

LOQ = 5 µg/kg

Thirty six sheep (18 males and 18 females) were administered a single oral dose of 2.265% oxfendazole suspension at a dose of 5.9 mg/kg BW. Two treated males and two treated females were slaughtered on days 10, 12, 14, 16, 18, 20, 22 and 24 post treatment. The residue concentrations of oxfendazole sulfone were below the limit of quantification in muscle, kidney and fat at all days post treatment. Liver contained 476, 292 and 127 µg/kg of oxfendazole sulfone at days 10, 12 and 14, respectively. At all other times, the concentration was near or below the limit of quantification (de Montigny, 1996b).

Sheep (milk)

A single dose of febantel 2.5% suspension was administered orally to 8 lactating sheep at 5.0 mg/kg BW. Milk samples were taken from the animals 3 days before and 5 days after administration. Two samples were collected at each day, one in the morning and one in the afternoon. The results of this study are summarized in Table 6 (Schmidt, 1995b).

Table 6. **Mean concentrations (µg/L) of oxfendazole sulfone in milk of sheep receiving 5.0 mg febantel/kg BW (n= 8 animals)**

Hours after administration	Milk	Hours after administration	Milk
0	<LOQ	72	20
10	357	82	15*
24	260	96	9**
34	158	106	<LOQ-11
48	73	120	<LOQ-7
58	42	130	<LOQ

*n = 7; **n = 6; LOQ = µg/L

Goats (milk)

Two groups of four goats each were dosed orally with fenbendazole as a paste suspension at 5 (1x the recommended dose) and 25 mg/kg BW. The concentration of fenbendazole was determined in milk at 2 hours and then at 12-hour intervals for 120 hours post-treatment. The detection limit of the method was 10 µg/L. Although the metabolites of fenbendazole were observed in the chromatograms, they were not quantified. The highest levels for both doses were observed at 12-hours post-dose. In these samples, fenbendazole concentrations were 98 ± 21 µg/L and 443 ± 213 µg/L for the 5 and 25 mg/kg doses, respectively. Fenbendazole levels were below the detection limit after 48 hours and 72 hours for the 5 and 25 mg/kg doses, respectively. The fenbendazole depleted from the milk with a 9.65 hour half-life (Waldhalm et al., 1989).

Pigs

Pigs (5 per groups) were treated orally with fenbendazole 1.5% pellets at a dose rate of 5 mg/kg BW. Using HPLC, the plasma levels of fenbendazole (FBZ) and its metabolites oxfendazole (FBZ-SO) and oxfendazole sulphone (FBZ-SO_2) were determined 4, 6, 8, 10, 12, 24, 72, 120 and 168 hours after oral administration. Combined residues, expressed as FBZ-SO_2, were determined in liver, kidney, muscle, fat and skin at 12, 24, 72, 120 and 168 hours after oral administration of the pellets. FBZ was rapidly absorbed reaching it highest concentrations in plasma (C_{max}) four hours after dosing and was also rapidly eliminated from the blood ($t_{1/2}$ = 5.5 hours). The two metabolites FBZ-SO and FBZ-SO_2 reached their C_{max} at 8 and 24 hours after administration, respectively. Measurable concentrations of FBZ and its metabolites (determined as FBZ-SO_2) were detected up to 24 hours after dosing in muscle, kidney, skin and fat and up to 72 hours in liver. The results of this study are summarized in Tables 7 and 8 (Schmid and Schmidt, 1996).

Table 7. **Mean plasma concentration (µg/L) of FBZ and its metabolites in pigs receiving 5 mg fenbendazole/kg BW**

Hours after treatment	FBZ	FBZ-SO	FBZ-SO_2	Hours after treatment	FBZ	FBZ-SO	FBZ-SO_2
4	145	622	33	24	28	959	370
6	128	948	76	72	0	14	0
8	106	1154	120	120	0	0	0
10	46	841	157	168	0	0	0
12	51	521	171				

Table 8. Mean tissue concentrations (µg/kg) and range (in parentbeses) of oxfendazole sulfone in pigs receiving 5 mg fenbendazole/kg BW

Hours after treatment	Liver	Kidney	Muscle	Skin*	Fat**
12	3160(2665-3790)	785(430-986)	809(660-1019)	975(753-1312)	1291(939-1808)
24	6317(2939-9990)	1086(809-1483)	918(657-1292)	923(634-1405)	910(753-1285)
72	18(5-63)	<LOQ	<LOQ	<LOQ	<LOQ
120	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
168	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

* skin plus subcutaneous fat **perirenal fat

Horses

Fenbendazole as a 10% suspension was orally administered to 16 borses at a dose rate of approximately 10 mg/kg bw daily for 5 consecutive days. Two untreated control animals were also included in the study. Two treated males and females were slaugbtered on each of days 5, 10, 15, and 20 following the first treatment and tissue samples were taken for analyses. In addition, blood samples from each animal were taken before dosing and at intervals during and after dosing period as follows: 0, 4, 6, 8, 16, 24, 32, 48, 56, 72, 80, 96, 100, 102, 104, 112, 120, 128, 144 hours and on day 9, 10, 11*, 12*, 15*, 20* and 25* (* if not slaughtered earlier).

The plasma analysis show, that during the multiple dosing of fenbendazole over a period of five consecutive days, all treated animals exhibit measurable concentration of parent administration on day 5, all three compounds were eliminated from blood very rapidly, within two or three days. The terminal half-lives of fenbendazole and of oxfendazole sulfone amount to approximately 9.5 hours and that of oxfendazole amounts to approximately 18.5 hours. The results of this study are summarized in Table 9.

The determinations of fenbendazole and its metabolites in borse tissue show that by 5 days after the last dosing (equivalent to 10 days after the first dosing) neither fenbendazole nor its metabolites could be measured in any of the tissues investigated in concentrations higher than the limit of quantification. All three compounds were very rapidly eliminated from the body of horses (Schmidt , 1997a).

Table 9. Mean plasma concentrations (µg/L) of 16 treated horses

Time (hours)	Study day	FBZ	FBZ-SO	$FBZ-SO_2$	Time (hours)	Study day	FBZ	FBZ-SO	$FBZ-SO_2$
0	1*	<LOQ	<LOQ	<LOQ	96	5*	57	97	89
4		42	35	55	100		251	82	93
6		40	43	61	102		186	117	111
8		43	60	69	104		262	83	111
16		31	58	45	112		148	59	82
24	2*	20	53	26	120	6	78	53	51
32		106	84	100	128		49	46	27
48	3*	47	57	56	144	7	14	23	<LOQ
56		136	94	98	192	9	<LOQ	<LOQ	<LOQ
72	4*	31	82	53	216	10	<LOQ	<LOQ	<LOQ
80		166	104	104					

*days of administration

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The separate analytical methods for the quantitative determination of residues of fenbendazole and its metabolites (oxfendazole and oxfendazole sulfone) in edible tissues (cattle, sheep, pigs and horses) and milk (cattle and sheep) have been combined in a single report (Schmidt, 1997b). The method can also be applied to the determination of fenbendazole and its metabolites in skin of pigs.

Fenbendazole and its two metabolites are extracted from milk and tissue homogenates using ethyl acetate. Fenbendazole and oxfendazole are oxidized to oxfendazole sulfone with peracetic acid. The total amount of oxfendazole sulfone is quantitatively analyzed after extensive purification using HPLC with fluorescence detection at 295 nm (Ex.) and 410 nm (Em.). Methyl-(5-cyclopentylsulfinyl-1H-benzimidazole-2yl)-carbamate is used as an internal standard. Non-interference from albendazole, mebendazole, ivermectin, levamisole, streptomycin and tetracycline was demonstrated. The method is linear in the range from 5 to 1000 µg/kg in liver and from 5 to 200 µg/kg in kidney, fat and muscle of all species investigated, as well as in skin from pigs. The linear range in milk from cattle and sheep ranges from 5 to 1000 µg/L.

The mean absolute recovery for fenbendazole and its metabolites (measured as oxfendazole sulfone) ranged from:

- 70.8% (muscle) to 87.8% (liver) in cattle;
- 48.8% (fat) to 93.9% (liver) in sheep;
- 54.1% (fat) to 102.9% (kidney) in pigs;
- 58.9% (fat) to 75.0% (liver) in horse;
- and from 94.8% (milk of cattle) to 95.6% (milk of sheep).

APPRAISAL

The Committee has previously considered the three anthelmintic agents febantel, fenbendazole and oxfendazole at the thirty-eighth and forty-fifth meetings. A group temporary ADI of 0 - 4 µg per kg of body weight was established for all three anthelmintics based on a NOEL of 0.7 mg per kg of body weight per day for oxfendazole identified at the thirty-eighth meeting and a safety factor of 200.

At the thirty-eighth meeting the Committee recommended group MRLs for each of the three anthelmintics using oxfendazole sulfone as the marker residue. Temporary MRLs, expressed as the sum of the three principle metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) calculated as oxfendazole sulfone equivalents, were recommended for cattle, sheep, and pigs: muscle, fat, and kidney, 100 µg/kg; liver, 500 µg/kg; milk (cow), 100 µg/L.

At the forty-fifth meeting several residue studies in cattle, sheep and pigs were reviewed. However, the residue-depletion studies on total residues of fenbendazole, oxfendazole and oxfendazole sulfone in cattle and sheep following the administration of febantel and oxfendazole were ongoing. In addition to the results from these studies, the Committee noted that, with the increasing production of goats in developing countries, residue data would be required for establishing MRLs in this species.

At the present meeting, the Committee reviewed the results of the residue depletion studies for febantel and oxfendazole in cattle and sheep as well as three new studies with fenbendazole, one study in the horse and two in pigs. Also, information on the metabolism, pharmacokinetics and residue depletion of fenbendazole and oxfendazole in goats, sheep and cattle were compared. In addition, a single method for all three drugs in milk and tissues was evaluated. The method measures the sum of the three principle metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) in both edible tissues and milk as equivalents of oxfendazole sulfone. The sponsor claimed that the limit of quantification (LOQ) of this method in all tissues and milk is 5 µg/kg.

Metabolism data

The *in vitro* oxidative metabolism of fenbendazole was studied using liver preparations in a number of species including cattle, sheep and goats. All species investigated produced the sulfoxide metabolite (oxfendazole) and upon further oxidation, the sulfone (oxfendazole sulfone) but at varying rates. Although some degree of species specific preference in the rates of formation of the two principle metabolites was seen, the differences were not of practical significance.

The disposition of fenbendazole was studied in the plasma, urine and feces of goats after oral and intravenous administration. As fenbendazole, oxfendazole and oxfendazole sulfone were the major drug-related constituents in plasma, the metabolism of fenbendazole in the goat was demonstrated to be similar to other species including cattle and sheep.

Pharmacokinetic data

The pharmacokinetics of oxfendazole in goats was compared to that in sheep. After intravenous administration of 7.5 mg oxfendazole/kg BW to sheep and goats, the areas under concentration-time curve (AUCs) of oxfendazole in the two species were not significantly different. Similarly, the total AUCs for the three metabolites were not significantly different. However, the bioavailability of oxfendazole in goats after oral administration was only about 42% of that in sheep.

The pharmacokinetics of a 4% powder fenbendazole formulation versus a 1.5% pellet formulation (dose rate 5 mg fenbendazole/kg BW) was determined in pigs (two-way crossover bioequivalence study). Comparing the mean pharmacokinetic parameters of all 12 pigs after administration either of the pellets or powder, the maximum concentrations (C_{max}), the times of maximum concentrations (T_{max}) and the AUCs of fenbendazole and oxfendazole were similar.

Residue data

All values for tissue residue concentrations in this section, except the study in goats with fenbendazole, were obtained by a method that determines the sum of the three principle metabolites calculated as the oxfendazole sulfone equivalent. The plasma values were determined by a method that measures the three metabolites individually.

Cattle A single dose of febantel 10% suspension was administered orally to 16 cattle at 7.5 mg/kg BW. At day 7, 14, 21 and 28 days post treatment 4 treated animals were sacrificed each time. The concentrations of oxfendazole sulfone in muscle and kidney were at or below the LOQ at all times. The concentrations in liver and fat were 115 and 19 µg/kg at day 7 and at or below the LOQ at all other times.

Thirty-two cattle were orally administered oxfendazole suspension at a single dose of 4.5 mg oxfendazole/kg BW. Edible tissues were collected from treated animals slaughtered on days 10, 12, 14, 16, 18, 20, 22 and 24 of the study. Residues of oxfendazole and its metabolites were undetectable in kidney and muscle after day 10. Mean levels of metabolites present in liver had fallen to less than 20 µg/kg by day 14 declining to less than 10 µg/kg by day 18. Levels in fat were less than 10 µg/kg on day 10 declining to undetectable levels by day 14.

A single dose of febantel 10% suspension was administered orally to 8 lactating cows at 7.5 mg/kg BW. Milk samples were taken from 12 animals (4 of them were untreated) beginning 3 days before to 5 days after administration. Two samples were collected at each day for each cow, one in the morning and one in the evening. Residues of febantel and its metabolites were a maximum of 268 µg/L at 34 hours after administration to near the LOQ at 96 hours.

Eight lactating cows were given a single oral dose of 9% oxfendazole suspension at 4.5 mg/kg BW. Individual milk samples were collected from each cow immediately prior to treatment and every 12 hours thereafter up to 120 hours post treatment. Residues of oxfendazole and its metabolites peaked at 222 µg/L at 24 hours and declined to near or below the LOQ at 96 hours.

Sheep A single dose of febantel 2.5% suspension was administered orally to 16 sheep at 5.0 mg/kg BW. At day 3, 7, 14 and 21 days post dose 4 treated animals were sacrificed at each time. Residue concentrations of febantel and its metabolites in muscle, liver, kidney and fat were 40, 4617, 199 and 133 µg/kg, respectively, at 3 days after administration. The residues deplete to near or below the LOQ in muscle, liver, kidney and fat by day 7, 21, 14 and 14, respectively.

Thirty-six sheep were administered a single oral dose of 2.3% oxfendazole suspension at 5.9 mg/kg BW. Two treated males and two treated females were sacrificed on days 10, 12, 14, 16, 18, 20, 22 and 24 post treatment. The residue concentrations of oxfendazole sulfone were below the LOQ in muscle, kidney and fat at all days post treatment. Liver contained 476, 292 and 127 µg/kg of oxfendazole sulfone at days 10, 12 and 14, respectively. At all other times, the concentration was at or below the LOQ.

A single dose of febantel 2.5% suspension was administered orally to 8 lactating sheep at 5.0 mg febantel/kg BW. Milk samples were taken from the animals beginning 3 days before to 5 days after administration. Two samples were collected

at each day from each sheep, one in the morning and one in the afternoon. The residue concentrations peaked at 357 µg/L at 10 hours and depleted to the LOQ by 106 hours.

Goat Two groups of four goats each were dosed orally with fenbendazole as a paste suspension at 5 mg/kg BW (the recommended dose) and 25 mg/kg BW. The concentration of fenbendazole was determined in milk at 2 hours and twelve-hour intervals for 120 hours post-treatment. The detection limit of the method was 10 µg/L. Although the metabolites of fenbendazole were observed in the chromatograms, they were not quantitated. The highest levels for both doses were observed at 12-hours post-dose. In these samples, fenbendazole mean concentrations were 98 µg/L and 443 µg/L for the 5 and 25 mg/kg doses, respectively. Fenbendazole levels were below the detection limit after 48 hours and 72 hours for the 5 and 25 mg/kg doses, respectively. The fenbendazole depleted from the milk with a half life of 9.65 hour.

Pig Pigs (5 per group) were treated orally with fenbendazole 1.5% pellets at a dose rate of 5 mg/kg BW. Combined residues, expressed as oxfendazole sulfone were determined in liver, kidney, muscle, fat and skin 12, 24, 72, 120 and 168 hours after oral administration of the pellets. Residue concentrations of fenbendazole and its metabolites peaked at 24 hours in liver, kidney and muscle: 6317, 1086 and 918 µg/kg, respectively. Residue concentrations in skin and fat peaked at 12 hours after treatment at 975 and 1291 µg/kg, respectively.

Horse Fenbendazole as a 10% suspension was orally administered to 16 horses at a dose rate of approximately 10 mg/kg BW daily for 5 consecutive days. Two treated males and females were slaughtered on each of days 5, 10, 15, and 20 following the last treatment and tissue samples were taken for analyses. The determinations of fenbendazole and its metabolites in horse tissue show that by 5 days after the last dosing neither fenbendazole nor its metabolites could be measured in any of the tissues investigated in concentrations higher than the limit of quantification.

Analytical Method

The separate analytical methods for the quantitative determination of residues of fenbendazole and its metabolites (oxfendazole and oxfendazole sulfone) in edible tissues (cattle, sheep, pigs and horses) and milk (cattle and sheep) have been combined in a single report. The method can also be applied to the determination of fenbendazole and its metabolites in skin of pigs.

Fenbendazole and its two metabolites are extracted from milk and tissue homogenates using ethyl acetate. Fenbendazole and oxfendazole are oxidized to oxfendazole sulfone with peracetic acid. The total amount of oxfendazole sulfone is quantitatively analyzed after extensive purification using HPLC with fluorescence detection. An internal standard is used to correct for recoveries. The method has a linear range of 5 to 1000 µg/kg in liver and from 5 to 200 µg/kg in kidney, fat and muscle of all species investigated, as well as in skin from pigs. The linear range in milk from cattle and sheep is 5 to 1000 µg/L. The range of mean absolute recovery for fenbendazole and its metabolites (measured as oxfendazole sulfone) in cattle, sheep, pigs and horse were 70.8% (muscle) to 87.8% (liver) in cattle; 48.8% (fat) to 93.9% (liver) in sheep; 54.1% (fat) to 102.9% (kidney) in pigs; 58.9% (fat) to 75.0% (liver) in horse; and 94.8% (milk of cattle) to 95.6% (milk of sheep).

Based on a statistical evaluation of the precision data of the method by the Committee for various species/tissues combinations and milk, the LOQ was found to vary from approximately 5 to 35 µg/kg.

Maximum Residue Limits

In reaching its decision on MRLs, the Committee took into account the following factors:

- An ADI of 0 - 7 µg per kg of body weight was established. This would result in a maximum ADI of 420 µg for a 60-kg human.
- Metabolism, pharmacokinetic and residue depletion information are similar between cattle, sheep, goats, pigs and horses.
- The correlation between plasma and milk residues are similar in sheep and cows.
- With the analytical performance data provided by the sponsor, the highest LOQ for any of the edible tissues or milk is well below the recommended MRLs.
- Residues are expressed as oxfendazole sulfone equivalents, tissues and milk in all species.
- The MRLs represent the sum of the three principle metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) calculated as oxfendazole sulfone equivalents.

The Committee recommended MRLs for febantel, fenbendazole and oxfendazole of 100 µg/kg (muscle, fat and kidney), 500 µg/kg (liver) in cattle, sheep, goats, pigs and horses and 100 µg/L in milk for cattle and sheep. The recommended MRLs would result in a theoretical maximum daily intake of 240 µg of residues based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 L of milk.

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GENTAMICIN

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ADDENDUM
to the gentamicin residue monograph
prepared by the 43rd meeting of the Committee
and published in FAO Food and Nutrition Paper 41/7
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INTRODUCTION

The Committee, at its 43rd meeting, stated that a validated chemical analytical method with a limit of quantification at or preferably below the temporary MRL of 0.1 mg/L recommended for milk was required for evaluation. Additional information with respect to the toxicological evaluation was also requested before the temporary ADI recommended by the 43rd meeting of the Committee could be replaced by a permanent ADI. Establishment of a permanent ADI would permit the replacement of the temporary MRLs assigned by the 43rd Meeting of the Committee with a recommendation for permanent MRLs, with the exception of the MRL for milk, which required the validated methodology requested.

ANALYTICAL METHOD

A high performance liquid chromatographic (HPLC) method for the quantification of residues of gentamicin in cattle milk, as well as in muscle, liver, kidney and fat of cattle and pigs and skin of pigs was presented for review (Stilzebach, 1996a,b). The method for tissues, except fat, includes extraction of the analyte into buffer, deproteinization by heating, with subsequent clean-up using a Sephadex column. A further clean-up on an SAX column is required for muscle and liver. Gentamicin residues are detected by fluorescence after pre-column derivatization with 9-fluorenylmethyl chloroformate (Fmoc-Cl), with excitation at 261 nm and emission at 313 nm. HPLC separation is on a reversed phase C-18 column using gradient elution with a mixture of water and acetonitrile. The method, when applied to fat or skin from pigs, omits the deproteination step. For milk, the initial extraction into buffer is omitted.

The four major components of gentamicin are usually designated as C_1, C_{1a}, C_2, and C_{2a}, but are identified in the method as G1, G2, G3, and G4. The method sponsor did not have authentic standards of the 4 major gentamicin components, so the identification G1 to G4 relates to order of elution and does not relate to the order C_1, C_{1a}, C_2, C_{2a}. The difference between the components of gentamicin is the degree of methylation or, in the case of C_2 and C_{2a}, in the location of the methyl group. It is very important to recognize that gentamicin is not a single entity. The ratio between the four major components is not constant between different batches and producers of gentamicin. In the report on the analytical method provided for review, the proportions of the different components in the analytical standard used in the study were not available.

Laboratories using this method will require authentication of their standard and should obtain samples of the four major gentamicin components for calibration. However, the four major peaks are separated, with retention times of 20 to 25 minutes, and quantification is by external standard. Validation included testing to determine accuracy (as recovery) and precision on fortified samples, the determination of limits of quantification and detection and testing for interferences. Due to interfering co-extractives, the gentamicin C_1 peak could not be used in the analysis of cattle muscle, liver and fat, while the gentamicin C_1 and C_2 peaks could not be used for the analysis of pig muscle and liver samples. Quantification was based on the remaining components. Limits of detection were based on the analysis of blank matrix and varied for the individual components. Recovery was determined at the limit of quantification (LOQ), and twice and 10 times the LOQ. The results of the validation are summarized in Table 1.

Table 1. **Performance characteristics of liquid chromatographic method of analysis for gentamicin residues in edible tissues of cattle and pigs and in cows' milk.**

Species	Tissue	LOQ* (mg/kg)	Lowest fortification level (mg/kg)	Recovery (%)	Coefficient of Variation (%)
cattle	muscle	0.10	0.10	90	13
	liver	0.20	0.20	95	7
	kidney	1.0	1.0	85	7
	fat	0.10	0.10	77	4
	milk	0.10	0.10	71	10
pig	muscle	0.10	0.10	81	4
	liver	0.20	0.20	72	5
	kidney	1.0	1.0	94	15
	fat	0.10	0.10	85	6
	skin	0.10	0.10	97	7

* LOQ = Limit of quantification, determined with respect to CVMP criteria, based on the lowest fortification level which meets the requirements for accuracy (as recovery) and precision.

APPRAISAL

Gentamicin was previously evaluated at the 43rd Meeting of the Committee. A temporary ADI of 0-4 μg/kg of body weight was established using a microbiological end-point and temporary MRLs were recommended of 100 μg/kg for muscle and fat, 200 μg/kg for liver and 1000 μg/kg for kidney in both cattle and pigs, as well as 100 μg/L for cows' milk, expressed as parent drug. The Committee requested the following information for evaluation in 1997:

1. Results of studies on the effects of gentamicin on specific genera of microorganisms obtained from the human intestine.

2. Additional data to assist in the assessment of carcinogenic potential, which should include:
   (a) results of genotoxicity assays for gene mutations in mammalian cells and chromosomal aberrations *in vitro* and *in vivo*; and
   (b) details of an investigation on possible structural similarities between gentamicin and known carcinogens.

3. A validated chemical analytical method with a limit of quantification below the MRL recommended for milk.

Pharmacokinetic data

No additional data were requested or provided.

Residue data

No additional data were requested or provided.

Analytical methods

Residue studies considered by the 43rd Meeting of the Committee primarily relied on microbial growth inhibition assays. Given the non-specificity of microbial growth inhibition assays and the apparent availability of liquid chromatographic assays for gentamicin residues in edible tissues, the Committee requested that a method based on a chemical assay be provided for the analysis of gentamicin residues in milk, with a limit of quantification below the MRL.

It was noted by the 43rd meeting of the Committee that while no analytical methods were available that met the multi-laboratory validation criteria described in Codex Alimentarius, Volume 3 (1993), there were published methods in the current scientific literature for gentamicin residue analysis in edible tissues based on high performance liquid chromatography. Several such methods were included in method compilations prepared for regulatory authorities.

An HPLC method for the quantification of residues of gentamicin in cattle milk, as well as muscle, liver, kidney and fat of cattle and pigs and skin of pigs was presented for review. The method for tissues, except fat, includes solvent extraction of the analyte into buffer, deproteination by heating, clean-up using solid-phase extraction and analysis by liquid chromatography. Detection is by fluorescence after pre-column derivatization. Deproteination is not required for the analysis of fat or skin from pigs, while the initial extraction into buffer is omitted for the analysis of cows' milk. The four major components of gentamicin (C_1, C_{1a}, C_2, C_{2a}) are separated, with retention times of 20 to 25 minutes, and quantification is by external standard. Interfering co-extractives prevent the use of all four components for quantitative analysis in some tissue matrices. A standard containing the four major components was used in this study. For quantification, the Committee considered that well-characterized standards, preferably of the 4 individual major gentamicin components, should be used for calibration in the analysis. The method was tested using samples fortified at the MRLs recommended by the Committee at its 43rd Meeting. Analytical recoveries were 72-97% for tissues and 71% at 0.1 mg/kg for cows' milk. The method appears suitable for use in a regulatory program to determine compliance with recommended MRL's for residues in edible tissues from cattle or pigs, or in cows' milk.

Maximum Residue Limits

In recommending MRLs, the Committee took into account the following factors:

- An established ADI of 0-20 µg per kg of body weight based on a microbiological endpoint derived from data provided for review by the present Committee. This would result in a maximum ADI of 1200 µg for a 60-kg person.
- Gentamicin residues are persistent in kidney and liver, but deplete rapidly in muscle, fat and milk.
- A suitable analytical method is available for analysis of gentamicin residues in edible tissues and milk. The LOQ for milk is 100 µg/L.
- The marker residue is parent drug.

On the basis of the maximum observed residues in studies with gentamicin in food animals presented for review by the 43rd Meeting of the Committee, the following permanent MRLs are recommended for edible tissues of cattle and pigs, expressed as parent drug:

Muscle	100 µg/kg
Liver	2000 µg/kg
Kidney	5000 µg/kg
Fat	100 µg/kg

The Committee also recommended a permanent MRL of 200 µg/L for gentamicin in milk from cattle.

The MRL's recommended above would result in a theoretical daily maximum intake of 785 µg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 L of milk.

REFERENCES

Codex Alimentarius, Volume 3 (1993). Residues of Veterinary Drugs in Foods, 2nd ed., Food and Agriculture Organisation of the United Nations, Rome, Italy.

Stilzebach, D. (1996a). Validation of a method for the quantification of gentamicin residues in matrices of cattle. ECON Project EF 95-12-01 Final Report, August 21, 1996, Sponsor Submitted.

Stilzebach, D. (1996b). Validation of a method for the quantification of gentamicin residues in matrices of pig. ECON Project EF 95-12-02 Final Report, August 23, 1996, Sponsor Submitted.

IMIDOCARB DIPROPIONATE

First draft prepared by
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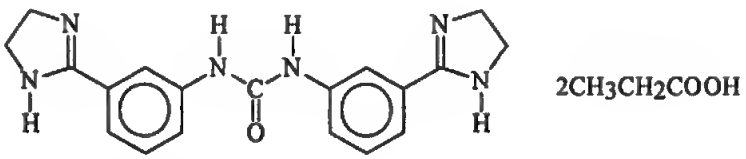
IDENTITY

Chemical name: N,N'-bis[3-(4,5-dihydro-1H-imidazol-2-yl)phenylurea dipropionate

Synonyms: Imizol; 4A65; BW4A65; HR-2073; IMDP

Structural formula:



CAS number: 27885-92-3 (imidocarb)

Molecular formula: $C_{25}H_{32}N_6O_5$

Molecular weight: 496.55

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Imidocarb (purity not specified).

Appearance: Off-white to pale cream powder.

Melting point: 254°C (Sponsor); 350°C (as hydrochloride salt, Merck Index)

Solubility: Soluble in water (74% m/V) and methanol; moderately soluble in acids; slightly soluble in buffer at pH 7.8; practically insoluble in base; insoluble in non-polar organic solvents, such as acetone.

Optical rotation: Optically inactive.

Ultraviolet maxima: Not reported.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Imidocarb has been approved in a number of countries since the early 1970's for the treatment of the protozoal diseases babesiosis (in cattle and sheep) and anaplasmosis (in cattle). Currently, it is used in Africa and the mid-East, Europe and South America for the treatment of these diseases, which are transmitted by ticks. The typical commercial

formulation is an injectable solution of 12% m/V imidocarb dipropionate in water buffered to pH 4.5 with propionic acid.

Dosage

The typical dosage for cattle is a single treatment of 1.2 to 3.0 mg/kg BW imidocarb dipropionate, which may be repeated at 4 week intervals for prophylaxis of babesiosis. A single dose of 1.2 mg/kg BW imidocarb dipropionate is recommended for sheep, with a second dose 2 weeks later, if required. The formulated product may be injected either by intramuscular (IM) or subcutaneous (SC) injection, with SC being the preferred route of administration.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics

Toxicological Test Species

Rats

Rats administered orally with ^{14}C-imidocarb dihydrochloride were killed at 2, 6 or 24 hours after dosing (Farebrother, 1973). In the same study, other rats dosed orally with ^{14}C-imidocarb dipropionate were killed at 48 hours after exposure, while rats treated subcutaneously with the dihydrochloride were sacrificed 7 days following dosing. Wholebody autoradiography, after sacrifice of the rats killed at 2 or 6 hours, showed activity outside the gut only in liver and kidney, with slight traces of radioactivity remaining in these organ tissues at 24 hours. In the rats killed 48 hours after oral treatment with ^{14}C-imidocarb propionate, traces of radioactivity were found in kidney tissue. In the rats killed at 7 days after treatment, high activity remained at the injection site, with radioactivity also detectable in skeletal muscle (higher near the injection site) and traces in kidney and liver. The results demonstrated poor absorption of imidocarb from the gut following oral administration, with more effective distribution resulting from the subcutaneous injection.

In the first of two studies using unlabelled imidocarb (Nimmo-Smith, 1968), a variety of doses and routes of administration were used, with the salt form of imidocarb not specified. Following SC injection at 10 mg/kg BW, only about 19% of the dose as parent compound was excreted within 78 hours, with three-quarters of this in the urine. Following multiple doses administered by the oral or intraperitoneal routes, highest tissue residues were found in kidney, with significant residues also found in liver. In rats treated by a single SC injection of 5 mg/kg BW and killed at varying time periods up to 56 days following treatment, residues in kidney and liver were found to have an initial half-life of about 3 days, increasing after the initial 3 days to 7-8 days. There was indication of significant tissue binding in liver. In the second of these studies (Thomson, 1975), female Wistar rats were administered imidocarb dipropionate at 5 mg/kg BW by stomach tube, either using a single treatment or once daily for 30 days. Rats were sacrificed 24 hours after final exposure and tissues were assayed by an unspecified analytical method. Residues in kidneys were approximately 10 mg/kg, while residues in liver were in the range of 1-2 mg/kg and in muscle <0.5 mg/kg, for animals which received both the single and multiple doses.

Mice

Two studies were reported in which mice received 1 mg/kg BW ^{14}C-imidocarb dihydrochloride by intravenous (IV) injection (Anonymous, 1968a, b). In the first of these studies, excretion was rapid, with residues observed in urine within 5 minutes of dosing. Within 96 hours, about 90% of the drug was excreted (56-65% in urine, 23-25% in faeces). In mice killed at 5 minutes or 8 hours after dosing, residues in liver were constant at 33% of the dose, while in kidneys residues diminished from 27% of dose to 6%. At 96 hours, 2.8% of the dose was found in liver and 0.6% in kidneys. In the second study, mice were sacrificed at 3.5 hours after IV injection, with 29% of the dose found in liver and 6.8% in kidney. Within 2 hours of dosing, 37% of the drug was excreted in urine and 95% of the radioactivity co-eluted with imidocarb in three different paper chromatography separation systems. Over 90% of the residues extracted from liver and kidney also co-chromatographed with parent imidocarb, indicating little metabolic conversion of the parent compound.

Dogs

In male beagle dogs, administered 5 mg/kg BW imidocarb (free base basis) dipropionate once daily by gavage for 30 days and killed at 24 hours after final treatment (Chesher *et al*, 1976), highest residues were found in liver (98 ± 37 mg/kg), followed by kidneys (7.3 ± 2.1 mg/kg) and muscle (<0.5 mg/kg). In mongrel dogs which received an

intravenous bolus dose of 4 mg/kg imidocarb dipropionate, the plasma half-life $t_{½}$ was reported as 207 ± 45 min, with approximately 80% of the dose eliminated within 8 h of treatment (Abdullah and Baggot, 1983).

Monkeys

In 5 female monkeys dosed orally once daily for 30 days with 5 mg/kg BW imidocarb dipropionate, residues in tissues collected at slaughter (time following final dose not stated) were 1.02 ± 0.23 mg/kg in liver, 1.07 ± 0.62 mg/kg in kidney and <0.5 mg/kg in muscle (Thompson, 1975b).

Metabolism in Food Animals

Cattle

In a preliminary study comprising three reports (Nimmo-Smith and Savage, 1973; Chesher *et al*, 1973; Chesher and Malone, 1973), 2 lactating and 4 non-lactating cows were administered 6 mg/kg BW ^{14}C-imidocarb dipropionate by intramuscular (IM) injection twice, with 14 days between the injections. Maximum concentrations in plasma were achieved within 30 minutes and initial excretion, primarily in urine, was rapid. The plasma half-life lengthened with time following treatment, being 45-55 days by 60 days after the second dose. Less than half the dose was excreted in the first week following treatment, with elimination also slowing with increasing time. Maximum residues were found in milk within 24 hours of treatment, with the terminal half-life in milk being about 15 days. Total residues were highest in injection sites, followed by liver, kidney and muscle. This work was not conducted to GLP standards.

Subsequently, a non-GLP study was conducted in which 9 calves each received 0.5 mg/kg BW imidocarb dipropionate by IV injection, following which blood samples were collected at 5, 15, 30 and 60 min, and at 2, 4, 8, 16 and 24 h (Nimmo-Smith and Savage, 1974; Chesher *et al*, 1974). The calves were subsequently killed in groups of 3 at 7, 30 and 60 days after administration of the drug. The experiment was repeated with another 9 calves which received imidocarb dipropionate as a pour-on mixture applied as a 5% solution to provide a dose of 30 mg/kg BW, with blood samples collected at 2, 4, 8, 16 and 24 h and at 2, 4 and 7 days post-dosing. Animals were killed on the same schedule as the calves which received imidocarb intravenously. In addition, 5 heifers received imidocarb dipropionate at 3 mg/kg BW by IM injection in the gluteal muscle and were killed at 172 days following treatment. After the IV dosing, plasma half-life over the first hour was 24 min, increasing to 3.2 h over the next 7 h and to 7.5 h from hours 8-24. Drug concentrations in plasma achieved a concentration of 2.2 mg/L at 5 min after treatment, declining to 0.75 mg/L at 1 h and to 0.02 mg/L at 24 h By comparison, dermal treatment resulted in a mean concentration of imidocarb in plasma of 0.01 mg/L at 2 h after dosing, with the maximum mean value achieved being 0.10 mg/L at 16 h. The highest individual result was 0.17 mg/L in one animal at 2 days following exposure, with a greater variability in imidocarb concentrations in plasma evident for the dermal exposure, but with an overall greater persistence.

The following concentrations of imidocarb were found in animals killed following IV dosing: day 7 - liver, 4.62-6.89 mg/kg; kidney, 6.01-24.9 mg/kg; muscle, not tested; day 30 - liver, 1.75-3.59 mg/kg; kidney, 3.26-4.55 mg/kg; muscle, not tested; day 60 - liver, 0.68-3.29 mg/kg; kidney, 1.63-3.13 mg/kg; muscle, 0.16-0.30 mg/kg. Following dermal exposure, residues were as follows: day 7 - liver, 1.60-4.75 mg/kg; kidney, 25.5-45.3 mg/kg; muscle, not tested; day 30 - liver, 1.84-3.00 mg/kg; kidney, 1.07-2.63 mg/kg; muscle, not tested; day 60 - liver, 1.29-1.52 mg/kg; kidney, 1.74-4.49 mg/kg; and muscle, 0.23-0.32 mg/kg. In the 5 heifers slaughtered 172 days following IM injection with 3 mg/kg BW imidocarb dipropionate, residues were detected as follows: liver, 1.11 ± 0.55 mg/kg; kidney, 4.18 ± 3.05 mg/kg; muscle, 0.13 ± 0.04; and fat, 0.04 ± 0.03 mg/kg. The results indicated considerable differences in residues in individual animals and no statistically significant differences in residue levels in tissues, with the possible exception of kidney at 30 days (P=0.05), when residues resulting from IV dosing and dermal exposure were compared. In this study, plasma and tissue samples were analyzed first by a colourimetric method with a claimed limit of detection of 0.1 mg/kg (Nimmo-Smith and Ince, 1969) and, where greater test sensitivity was required, were subsequently analyzed by a fluorometric method (Nimmo-Smith and Norton, 1973).

A more recent study (Ferguson, 1996) was reported in which dairy cows and male and female calves were treated with a single SC dose at 3 mg/kg BW of ^{14}C-imidocarb dipropionate formulated as Imizol. Peak concentrations of 1.32 ± 0.44 mg equivalents/kg of ^{14}C-imidocarb were found in blood at 1 h post-treatment, remaining constant to 4 h, and then declining to <0.05 mg/kg by 3 days after treatment. In blood samples collected at 1, 6, 14 and 36 hours post-dosing, from 72.0 to 90.9% of the radioactivity recovered was protein bound, with no difference found at the various collection times. Excreta were collected for 28 days following treatment, with 58.52 ± 5.23% of the drug recovered, the majority (38.65 ± 4.63%) in faeces and the remainder (15.31 ± 3.20%) in urine. Most of the recovered radioactivity in urine was parent compound, but some faecal samples contained a significant amount of an unidentified metabolite (day 4, 27.86%; day 10, 13.22%).

In dairy cows slaughtered 28 days post-dosing and in calves killed at 56 and 90 days post-dosing, 77.72 ± 5.98% to 83.58 ± 4.94 % of the total radioactive residue in liver was recovered, of which 83 - 84% co-chromatographed with imidocarb by HPLC. The remaining recovered residue was contained in a smaller, unidentified chromatographic peak, which was also seen in other matrices. TLC analysis gave similar results. The minor peak observed in the day 28 samples was also seen in liver samples in the day 56 and day 90 groups. Over 90% of the total radioactive residue in kidneys was extractable in the day 28, 56 and 90 samples, with 80% or more of this matching imidocarb parent compound in HPLC (>90% at days 56 and 90). In muscle, 79.7% of total radioactive residues was recovered by extraction from the dairy cattle (day 28), with 90.6% recovered from day 56 samples and 96.0% from day 90 samples. Most of this was associated with parent compound in all muscle samples. An unidentified peak which accounted for 9.06 ± 4.98% of the total residues in liver in the day 28 samples was also present in the liver samples for days 56 and 90 and in all kidney samples. This compound, also found in faecal samples, was not detected in any muscle samples. In milk, 77.1 to 95.1% of total radioactivity was extracted, with the recovery increasing with time from dosing, and >70% of this being parent compound through day 3 samples. On day six 36% of the recovered radioactivity was parent compound and, due to the tow tevet of radioactivity recovered from subsequent samples, no assignment was made. The day 1 sample was the only one to contain a second compound, which accounted for 2.19% of the total radioactivity.

In another recent study (Coldham *et al*, 1995), no metabolism was seen for imidocarb in *in vitro* studies with bovine liver. Following a single SC injection of 3 mg/kg BW imidocarb dipropionate in cattle also reported in this study, depletion of imidocarb in tissues followed a two compartment model, with α- and β-phase halflives of 31.7 and 48.5 days in liver and 34.9 and 120.7 days in muscle, respectively.

Sheep

In a series of non-GLP experiments in which sheep were dosed with imidocarb dipropionate, two sheep killed 24 h after treatment with 50 μg/kg BW of ^{14}C-imidocarb had residues distributed throughout the central nervous system (Aliu *et al*, 1977). In three sheep which received 2.0 mg/kg BW imidocarb dipropionate intravenously, concentrations of imidocarb in plasma declined rapidly in the first hour from 10.8 μg/mL to 1.9 μg/mL and to <t μg/mL over the next 4 h. Imidocarb dipropionate administered by IM injection (4.5 mg/kg BW) to 7 sheep gave peak concentrations in plasma of 7.9 μg/mL within 4 h, decreasing to 4.6 μg/mL at 6 h. Subsequent reduction of residues was slow, following first-order kinetics, with detectable residues in plasma 4 weeks after treatment. As in studies with cattle, significant protein binding was observed. Initial urinary excretion rates were high (14.5% in first 24 h), but reduced rapidly (1.14% in second 24 h). Concentrations of imidocarb in the bile exceeded those found in plasma. No metabolites were found by TLC analysis of urine, bile, liver or kidney samples. Highest tissue residues were found in kidneys of 5 sheep slaughtered at 4, 6, and 24 h and 32 days, respectively after treatment with a single IM dose of 4.5 mg/kg BW imidocarb (2 sheep killed at 24 h, 1 only at other times). Residue concentrations were lower in liver, followed by fat and muscle. Imidocarb residues in milk of lactating ewes peaked at 12 hours post-dosing, decreasing slowly at about the same rate as residues in plasma.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Cattle

Total residues in tissues resulting from treatment of dairy cows and male and female calves with a single SC dose at 3 mg/kg BW of ^{14}C-imidocarb dipropionate formulated as Imizol (Ferguson, 1996) are reported in Table 1. In injection sites, mean residue values were as follows: day 28, 1.73 ± 1.20 mg/kg; day 56, 1.35 ± 1.70 mg/kg; day 90, 0.54 ± 0.30 mg/kg. Samples were analyzed initially by combustion to determine total ^{14}C-residues. Subsequently, total extractable residues, extracted into acetonitrile, followed by extraction at acid and basic pH's, were determined. The extractable residues were analyzed by liquid chromatography, with radiometric detection, to determine the amount of parent compound present in the extract. This measurement was undertaken for kidney and liver samples from each animal, as well as for pooled muscle and milk samples, for each time point. The determination was not made for fat, due to the relatively low amounts of residue found in that tissue. Furthermore, it should be noted that this extraction did not include the treatment with protease to release bound residues, which is included in the proposed regulatory method. These data, however, permit an estimation of the marker residue as a percentage of the total residue at the various time points included in the study.

Based on the data in Table 1, parent compound comprises, on average, 68% of the total ^{14}C-imidocarb residues found in liver at the various time points, 88% of the residue in muscle and kidney and 77% of the residue found in milk. There is no indication from the data that the proportion of the parent compound to total residues found in tissues or milk changes with time from treatment. In the case of milk, the data for the sample collected at 6 days were not included in the calculation of the average as the measurements were essentially at the detection limit and therefore were not considered as quantitatively reliable. They are included in the table, however, to demonstrate the elimination of residues from milk at 6 days post-treatment.

Table 1. **Total residues and residues of parent compound found in tissues and milk of dairy cattle[1] and calves[2] which received a single SC dose (3 mg/kg BW) of ^{14}C-imidocarb dipropionate.**

Sample	Day	Total ^{14}C-Residue (mg/kg)	% Total Residue Extractable	^{14}C- Imidocarb Residue as Parent (mg/kg)	Parent as % of Total Residue
Liver	28	8.24±4.07	78	5.34±2.35	66
	56	4.01±0.42	84	2.79±0.45	70
	90	2.19±0.83	81	1.51±0.76	67
Kidney	28	12.81±4.65	95	10.59±4.36	82
	56	3.77±0.93	95	3.44±0.64	92
	90	1.40±0.49	92	1.27±0.46	91
Muscle	28	0.68±0.80	80	0.54[a]	79
	56	0.41±0.22	91	0.37[a]	89
	90	0.31±0.18	96	0.30[a]	95
Fat	28	0.13±0.07	---	---	---
	56	0.10±0.02	---	---	---
	90	0.03±0.02	---	---	---
Milk	1	0.37±0.22	77	0.26[a]	70
	2	0.19±0.05	80	0.15[a]	79
	3	0.10±0.04	86	0.07[a]	73
	6	0.03±0.01	95	0.01[a]	36

[1] Treated animals were mature dairy cattle, weight range 470 to 575 kg on receipt (n = 6).
[2] Treated animals were calves, weight range 118 to 158 kg on receipt (n = 4 per group).
[a] Analysis of pooled samples.
--- Not analyzed.

Other Residue Depletion Studies (with Unlabelled Drug)

Cattle

In a study conducted to GLP standards, forty calves were administered a single SC dose of 3 mg/kg BW imidocarb dipropionate and slaughtered in groups of four at 14, 28, 42, 56, 70, 98, 140, 168, 196 and 224 days post-treatment (Gaffney, 1992). Muscle samples were initially analyzed by an HPLC method with a claimed limit of determination of 0.025 mg/kg and recovery of 68.7 ± 7.9%. Subsequently, muscle and liver samples were apparently analyzed by an HPLC method with a claimed limit of determination of 0.01 mg/kg and recovery of 90 ± 3% in muscle and, for liver, a limit of detection of 0.07 mg/kg and recoveries of 64 ± 4% (Coldham *et al*, 1995). Kidney, fat and injection site samples were also collected, but no analytical results were reported. Results of the muscle and liver analyses are given in Table 2. Analytical results reported for muscle in the second report (Coldham *et al*, 1995), differ slightly from those reported in the original research document, but the analytical conditions reported in this reference suggest that the samples were re-analyzed for the published paper. The elapsed period from sample collection to analyses as reported in this paper was not stated, so the reliability of using these results to predict residues in freshly collected tissue may be questioned.

Two older studies were also reported in which tissue residues were measured in cattle treated with imidocarb, but these studies were not to GLP standards. In the first study, the results of which are contained in two reports (Crawley and Thomas, 1981; Taylor *et al*, 1981), cattle were treated by IM injection with 1 or 2 doses of 3 mg/kg BW imidocarb dipropionate. Cattle which received the single dose were killed, in groups of four (3 treated, 1 control), at 7, 14 and 28 days post-dosing. Cattle, which received a second dose 28 days after the first dose, were slaughtered at 14, 28 and 42 days after the second dose. Liver, kidney, muscle, fat and injection sites were collected from all animals and analyzed by a colourimetric method of analysis (Nimmo-Smith and Ince, 1969). Residues found in tissues from the animals in this study are reported in Table 3.

Table 2. Residues of imidocarb in muscle and liver of calves dosed with a single SC injection of imidocarb dipropionate at 3 mg/kg BW (results corrected for recovery)

Days after dosing	Imidocarb residues in tissue (mg/kg), n = 4			Days after dosing	Imidocarb residues in tissue (mg/kg), n = 4		
	muscle[1]	muscle[2]	liver[2]		muscle[1]	muscle[2]	liver[2]
14	1.07 ± 0.39	1.05 ± 0.31	5.4 ± 0.61	98	0.21 ± 0.03		
28	0.38 ± 0.09			140	0.15 ± 0.05		
42	0.40 ± 0.09			168	0.10 ± 0.03		
56	0.37 ± 0.08			196	0.12 ± 0.02		
70	0.31 ± 0.11			224	0.06 ± 0.01	0.06 ± 0.02	0.12 ± 0.01

[1] Results as per Gaffney, 1992; [2] Results as per Coldham *et al*, 1995.

Table 3. Residues of imidocarb resulting from IM injection (1 or 2 doses) of 3 mg/kg BW imidocarb dipropionate in cattle

Withdrawal Time (days)	Number of injections	Range of Imidocarb Residues in Tissues, mg/kg (n=3)					
		Liver	Kidney	Muscle	Fat[1] (omental)	Initial Injection Site	Second Injection Site
7	1	13.6 - 19.8	9.0 - 20.1	0.5 - 2.2	<0.1	3.8 - 4.4	
14	1	7.3 - 11.0	8.4 - 10.7	0.5 - 2.1	0.2 - 0.4	1.5 - 3.4	
14	2	6.9 - 19.5	13.9 - 26.2	2.0 - 3.6	0.4	2.1 - 3.1	2.1 - 8.2
28	1	2.4 - 4.8	2.3 - 3.1	<0.1 - 0.8	<0.1	1.0 - 2.8	
28	2	8.9 - 21.3	15.1 - 22.9	1.0 - 1.9	0.4	1.1 - 2.5	1.6 - 2.6
42[2]	2	5.4, 8.2	6.5, 15.1	0.4, 0.8	0.3, 0.3	1.0, 1.6	0.6, 1.1

[1] Perirenal fat was also analyzed, but residues (0.1 mg/kg) were only found in one sample from one animal at each of days 7 and 14;
[2] Only two animals slaughtered in this group. (1), (2) indicates initial and second injection sites.

In the remaining study (Piercy and Malone, 1976), the persistence of imidocarb at intramuscular and subcutaneous injection sites in male calves which received 5 mg/kg BW imidocarb dipropionate was determined at 30 days following administration using the same colourimetric method as in the preceding study. Residues ranged from 3.46 - 5.35 mg/kg in the SC injection sites and from 1.75 - 3.90 mg/kg in the IM injection sites. There was little difference in the total residues found at the injection site for either type of administration (total residue 0.59 ± 0.09 mg for SC sites; total residue 0.64 ± 0.18 for IM sites).

Two studies were conducted to determine the depletion of imidocarb in milk, again not to GLP requirements. In the first of these studies, detailed in four reports (Crawley, 1982a; Crawley, 1982b; Taylor and Mountford, 1981; Taylor, 1981), 3 cows received a single dose of 3 mg/kg BW imidocarb dipropionate (Crawley, 1982a), which was repeated 28 days later (Crawley, 1982b). Samples were initially analyzed by a gas chromatographic method using alkali flame

detection (Taylor, 1981), but were subsequently analyzed by gas chromatography/mass spectrometry (GC/MS), with a limit of detection of 0.01 mg/L (Crawley, 1982a,b). Residue depletion results are given in Table 4.

In the second study, detailed in two reports (Crawley and Swallow, 1983; Woollon, 1983), 4 cows were treated in a crossover dosing regimen, repeated after 35 days, in which they received 3 mg/kg BW imidocarb dipropionate IM either in the cervical or gluteal musculature. Milk samples were collected for 8 days following treatment and analyzed as by gas chromatography/mass spectrometry (Crawley, 1982b). Peak residue levels were in samples collected 24 h following treatment (IM in cervical musculature, 0.38-0.73 mg/L; IM in gluteal musculature, 0.28-0.86 mg/L), declining by approx. 50% by day 2 and to 0.02 mg/L or less after 8 days. The depletion profile was similar for injection in either muscle site and consistent with results observed in a recent study where ^{14}C-imidocarb dipropionate was administered SC at 3 mg/kg BW (Ferguson, 1996).

Table 4. Depletion of Imidocarb in milk following IM dosing of lactating cattle with 3 mg/kg BW imidocarb dipropionate (1 or 2 treatments, 28 days apart), as measured by GC/MS.

Time Post-Dose (Days)	Range of Imidocarb Residue Concentration, mg/L[a] (n=3)	Time Post-Dose (Days)	Range of Imidocarb Residue Concentration, mg/L[a] (n=3)
0.5	0.30 - 0.66	21	<0.01
1.0	0.60 - 0.79	28	<0.01
2	0.20 -0.55	29 (2nd dose after day 28 sample)	0.35, 0.52[1]
3	0.07 - 0.23	30	0.11[2]
7	<0.01	31	0.07 - 0.30
14	<0.01	38	<0.01, 0.10[1]

[a] Results are mean values for milk from each animal
[1] Samples available from only 2 of 3 animals.
[2] Sample available from only 1 of 3 animals.

Sheep

In a non-GLP study, twelve female sheep received two IM injections at 1.2 mg/kg BW imidocarb dipropionate, with a 7-day interval between injections. Groups of 3 sheep were killed at 7, 14, 28 and 56 days after the second treatment. In addition, 3 sheep were injected only with water and slaughtered 28 days later to provide controls (Woollon and James, 1983). However, analytical results were reported only for tissues from the sheep killed at 7, 14 and 28 days after the second treatment (Crawley, 1983; McHardy *et al*, 1986). As in several other studies, the colourimetric method of analysis (Nimmo-Smith and Ince, 1969) was used for sample analysis. The report does not state if the analytical results, given in Table 5, were corrected for recovery.

Table 5. Residues of imidocarb in sheep, which received two IM doses, at a 7-day interval, of 1.2 mg/kg BW imidocarb dipropionate.

Withdrawal Time (days)	Range of Imidocarb Residues, mg/kg (n=3)					
	Liver	Kidney	Muscle	Fat	Initial Injection Site	Second Injection Site
7	5.7 - t4.3	22.6 - t21.2	t.t - t.2	<0.1 - 0.1	0.7 - 2.3	6.0 - 7.5
14	3.8 - 9.3	26.1 - 94.7	0.4 - 0.7	<0.1	<0.1 - 0.9	1.2 - 1.6
28	0.9 - 3.1	5.6 - 9.6	<0.1 - 0.4	<0.1	<0.1	0.2 - 1.0

In an earlier non-GLP study (Aliu *et al*, 1977), five sheep were injected IM with an aqueous solution of imidocarb dipropionate at a dose of 4.5 mg free base/kg BW. Individual sheep were killed at 4 h, 6 h and 32 days after dosing and two sheep were killed at 24 h after dosing. Tissue samples were analyzed using a spectrophotometric method described in the publication, with an estimated limit of detection of 5 mg/kg. The tissue distribution was similar to that observed

in the above study, bul values are not cited due to the small number of animals (1 or 2) for each timepoint. Milk samples were also analyzed in this study (estimated limit of detection of 1 mg/L), with residues ranging from 4.5 - 5.6 mg/L in the 4 - 24 h samples. Again, since the dose was above the recommended level and the sample numbers very small, few conclusions can be drawn from the study.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Analytical methods used in the early residue depletion studies were based on colorimetric (Nimmo-Smith and Ince, 1969; Aliu *et al*, 1977) or fluorometric (Nimmo-Smith and Norton, 1973) detection. Analytical sensitivity of these methods was limited in comparison to chromatographic techniques commonly used loday in residue analysis and validation was not adequate by current standards. In particular, method specificity was poorly defined. These methods would not be suitable for use in a residue control program.

The first chromatographic method described used GC with alkali flame detection of imidocarb residues in milk after acid hydrolysis, diazotization and iodination (Taylor, 1981). However, the reliability of results generated with this method was not considered satisfactory, with the subsequent development of a GC/MS method which, while improving analytical reliability, still required the rather cumbersome hydrolysis and derivatization procedures (Crawley, 1982a).

More recent GLP studies have used analytical methodology based on HPLC with UV-detection, with the initial validation being for bovine muscle tissue (Gaffney, 1992). Muscle samples were homogenized in TRIS buffer, after which *Subtilisin Carlsberg* was added to release bound residues. Following incubation, the samples were acidified lo precipitate protein, centrifuged and the supernatant was removed and made basic. Following removal of co-extractives with organic solvent, the aqueous extract was analyzed by HPLC under isocratic conditions on a cyano-bonded reverse phase column, with detection at 245 nm. Under the conditions of analysis, imidocarb eluted in 9 to 11 minutes, with nearly baseline resolution from co-extractives. Data demonstrated that imidocarb residues were apparently stable in muscle tissues stored at –20°C for up to a year. Subsequently, the method was modified to use a C-18 analytical column and applied to the analysis of bovine liver and muscle (Coldham *et al*, 1994; Coldham *et al*, 1995). Similar methodology was subsequently reported (Ferguson, 1996), with recovery data provided on fortified samples of bovine liver, kidney, muscle, fat and milk. Data generated in this study suggest some reduction in recovery of spiked imidocarb residues may occur in liver stored at –18°C for 54 days. Analytical results for fortified samples were: at a fortification level of 0.3 mg/kg; day 0, 0.24 mg/kg; day 54, 0.22 mg/kg; at a fortification level of 2.0 mg/kg; day 0, 1.76 mg/kg; day 54, 1.45 mg/kg. Performance characteristics reported for this method are summarized in Table 6.

Table 6. Performance characteristics of the liquid chromatographic assay for imidocarb residues in beef tissues and milk

Performance Characteristics	Liver	Kidney	Muscle	Fat	Milk
LOD (mg/kg)[1]	0.02	0.02	0.02	0.02	0.01
LOQ (mg/kg)	0.10	0.10	0.05	0.05	0.01[3]
Recovery (%)[2]	83.5	92.6	84.1	95.9	87.2
Precision (%)[2]	4.6	5.1	9.2	7.8	14.7

[1] The lowest calibration point having a signal-to-noise ratio greater than 3.
[2] Means of duplicate samples at 5 concentrations.
[3] The lowest concentration at which acceptable recovery was obtained.

An alternative approach has also been reported (Tarbin and Shearer, 1992), in which imidocarb residues in bovine kidney are determined following extraction in acetone under basic conditions, partitioning with chloroform, saturated aqueous salt and 40% sodium hydroxide, then clean up on a weak cation-exchange (carboxylic acid) solid phase extraction cartridge. The HPLC analysis on a C-18 column requires mobile phase switching and a column with a packing which is stable under various conditions of mobile phase pH. Total HPLC run time, including re-equilibration of the column, is 30 minutes. Detection is at 260 nm, with recoveries in the 75% range at 0.05 to 0.10 mg/kg and a claimed limit of detection of 0.001 mg/kg.

The HPLC methods described above appear suitable for regulatory use, although additional validation for appropriate species/matrix combinations is required. In addition, substitution of highly chlorinated solvents, such as chloroform, may be required.

APPRAISAL

Imidocarb had not been previously evaluated by the Committee.

Imidocarb is an anti-protozoal drug which has been used since the 1970's for the treatment of the protozoal diseases babesiosis in cattle and sheep and anaplasmosis in cattle. The preferred route of administration is by subcutaneous injection, but intramuscular injection may also be used.

Pharmacokinetic data

Rats Non-GLP pharmacokinetic studies using ^{14}C-imidocarb dipropionate and ^{14}C-imidocarb dihydrochloride were conducted in rats. In rats which received either of the imidocarb salts orally, absorption was poor, while subcutaneous injection with the dihydrochloride resulted in high residues at the injection site 7 days following treatment, with trace residues detectable in liver, kidney and muscle. In a non-GLP study, rats were dosed with unlabeled imidocarb at 10 mg/kg BW. Only about 19% of the dose, as parent compound, was excreted within 78 hours, with three-quarters of this in the urine. Multiple doses administered to rats by the oral or intraperitoneal route yielded highest tissue residues in kidney, followed by liver. A single SC injection of 5 mg/kg BW in rats revealed an initial half-life of residues in kidney and liver of about 3 days, increasing subsequently to 7-8 days. There was indication of significant tissue binding in liver. Administration of imidocarb dipropionate at 5 mg/kg BW by stomach tube, either using a single treatment or once daily for 30 days, resulted in residues in kidneys of about 10 mg/kg, while residues in liver were in the range of 1-2 mg/kg and in muscle were 0.5 mg/kg.

Mice Several non-GLP studies were also reported in mice. When mice were administered ^{14}C-imidocarb dihydrochloride by IV, excretion was rapid, with residues appearing in urine within 5 minutes of dosing and 90% of the drug was eliminated within 96 h (55-65% in urine and 23-25% in faeces). In mice sacrificed 3.5 h following IV administration of ^{14}C-imidocarb dihydrochloride, 29% of the dose was found in liver and 6.8% in kidney, with over 90% of the residue found in each tissue being parent compound. Parent compound also accounted for 95% of the residue found in urine.

Dog, monkey In a non-GLP study, dogs were administered 5 mg/kg BW imidocarb dipropionate once daily by gavage for 30 days and sacrificed at 24 hours after final treatment. Imidocarb was distributed as follows: liver, 98 mg/kg; kidney, 7.3 mg/kg; and muscle, <0.5 mg/kg. In a separate non-GLP study, the plasma half-life of imidocarb in dogs given an intravenous bolus dose of 4 mg/kg imidocarb dipropionate was 207 min, with approximately 80% of the dose eliminated within 8 h of treatment. In a non-GLP study using monkeys dosed orally once daily for 30 days with 5 mg/kg BW imidocarb dipropionate, imidocarb distribution in tissues was: liver, 1.02 mg/kg; kidney, 1.07 mg/kg; and muscle, <0.5 mg/kg.

Cattle Pharmacokinetic studies were conducted in cattle using ^{14}C-labeled and unlabeled imidocarb dipropionate. In a recent GLP study, cattle that were administered a single SC dose of 3 mg/kg BW of ^{14}C-imidocarb dipropionate had C_{max} of 1.32 mg/kg in blood within one hour of treatment. The concentration remained constant for 4 h, then declined to <0.05 mg/kg over the following 3 days. From 72-91% of the drug in blood was protein bound. Only 58% of the administered dose was eliminated over 28 days after treatment, distributed between faeces and urine in an approximately 3:1 ratio. Parent compound accounted for most of the residue in urine, but up to 28% of the residue found in faecal samples at 4 days post-dosing was an unidentified metabolite. The same metabolite accounted for 13% of the total residue in day 10 faecal samples, but was not present in samples tested at days 2 and 6. In tissues, the extractable portion without using enzymatic digestion of the total radioactive residue was: in liver, 81%; kidney, 94%; muscle, 89%; and milk, 81%. Parent imidocarb, as a fraction of total residue, was: liver, 68%; kidney, 88%; muscle, 88%; and milk, 77%. There was no apparent reduction in the proportion of parent compound to total residue observed for the various sampling dates. Other components present in extracts accounted for <10% of the total radioactive residue, indicating that metabolism is not significant. Tissue binding is most significant in liver, from which also the lowest proportion of the residue recovered is parent compound. This study confirmed findings of earlier non-GLP investigations where [^{14}C]imidocarb dipropionate was administered IM to cattle. A recent *in vitro* study using bovine liver gave no indication of any metabolism of imidocarb in this tissue.

In non-GLP studies in which calves received unlabeled imidocarb dipropionate intravenously or as a pour-on, distribution and elimination patterns were similar to those found in the recent GLP study reported above.

Sheep In several non-GLP studies conducted in sheep, IM administration of [^{14}C]imidocarb dipropionate resulted in distribution of residues throughout the central nervous system. Following IM injection at 4.5 mg/kg BW, C_{max} was 7.9 mg/L at about 4 hrs, after which concentrations declined slowly over 4 weeks to <0.1 mg/L, following first order kinetics. Significant protein binding was observed in plasma. There was no evidence of metabolite formation in urine, bile, liver and kidney samples. Urinary excretion was high within 24 h of treatment, declining rapidly afterwards.

Residue data

Cattle In dairy cattle and 9-month-old calves which received a single SC dose (3 mg/kg BW) of a formulation containing ^{14}C-imidocarb dipropionate, total residues in tissues (determined by combustion), collected at indicated withdrawal times, are reported in Table 7. These results demonstrate that elimination of residues is slow in all edible tissues. Total residues in milk reached a maximum 24 hrs following treatment (0.37 mg/L), declining to 0.10 mg/L at day 3 and to 0.02 mg/kg at day 8 and subsequent milking times through day 14.

Table 7. Total residues from a single SC dose of 3 mg/kg BW ^{14}C-imidocarb dipropionate to cattle.

	[^{14}C] -Imidocarb residues in tissues (mg/kg)				
Days Post-treatment	**Liver**	**Kidney**	**Muscle**	**Fat**	**Injection Site**
28 (6 cows)	8.24	12.81	0.68	0.13	1.73
56 (4 calves)	4.01	3.77	0.41	0.10	1.35
90 (4 calves)	2.19	1.40	0.31	0.03	0.54

In cattle treated with unlabeled imidocarb dipropionate (SC injection at 3 mg/kg BW), residues of parent compound in muscle tissue declined from 0.38 mg/kg at day 28 to 0.37 mg/kg at day 56 and to 0.21 mg/kg at day 98. Residues were still detectable in muscle (0.06 mg/kg) in animals slaughtered 224 days following treatment. Residues of parent compound in liver declined from 5.4 mg/kg at day 14 to 0.12 mg/kg at day 224. These tissue residue data are consistent with findings in earlier studies in which unlabeled imidocarb dipropionate was administered by IM or SC injection at various rates and in single or multiple doses. In two early non-GLP studies dairy cattle were treated by IM injection with imidocarb dipropionate at 3 mg/kg BW. Residues of parent compound in milk peaked at one day following treatment (0.60-0.79 mg/L), declined to 0.07-0.23 mg/L at day 3 and were <0.01 mg/L at days 7-28 following treatment. Milk samples, collected following administration of a second 3 mg/kg BW dose, gave similar depletion results after the day 28.

Sheep Several non-GLP studies were reviewed, including one in which 12 sheep received two IM injections at a rate of 1.2 mg/kg BW imidocarb dipropionate, with a 7-day interval between injections. At 7 days following the second treatment, residues in kidney ranged from 22.6 to 121.2 mg/kg, declining to 5.6-9.6 mg/kg at day 28. Residues in liver were from 5.7 to 14.3 mg/kg at day 7 and from 0.9 to 3.1 mg/kg at day 28, while in muscle, 1.1-1.2 mg/kg imidocarb was present at day 7, but <0.1 to 0.4 mg/kg remained at day 28. Residues in injection site muscle were higher than in normal muscle, but below residues in kidney and liver, for all sampling dates.

In 3 lactating sheep which received imidocarb dipropionate IM at 4.5 mg/kg BW, residues in milk ranged from 4.5 to 5.6 mg/L in samples collected from 4 to 24 h following treatment. The small numbers of animals involved and the methods and rates of dosing used limits the value of these studies in assessing residues.

Analytical methods

Recent GLP studies have used analytical methodology based on HPLC with UV-detection at 245 nm following treatment of tissues with enzymatic digestion to release residues, extraction, solvent partitioning, then clean-up by solid phase extraction. The performance characteristics of the method as reported by the sponsor are given in Table 8.

Table 8. **Performance characteristics of the liquid chromatographic assay for imidocarb residues in beef tissues and milk**

Performance Characteristic	Liver	Kidney	Muscle	Fat	Milk
LOD (mg/kg)[1]	0.02	0.02	0.02	0.02	0.01
LOQ (mg/kg)	0.10	0.10	0.05	0.05	0.01[3]
Recovery (%)[2]	83.5	92.6	84.1	95.9	87.2
Precision (%)[2]	4.6	5.1	9.2	7.8	14.7

[1] The lowest calibration point having a signal-to-noise ratio greater than 3.
[2] Means of duplicate samples at 5 concentrations.
[3] The lowest concentration at which acceptable recovery was obtained.

An alternative approach for the analysis of bovine kidney has also been reported using a weak cation-exchange solid phase extraction cartridge, followed by HPLC analysis on a C-18 column with UV-detection at 260 nm. This method requires mobile phase switching and a packed column that is stable under various conditions of the pH of the mobile phase, but should be within the capabilities of a typical residue laboratory. This method has the disadvantage that it includes a partitioning step with chloroform, which has been categorized as an ozone-depleting solvent. Recoveries are approximately 75% at 0.05 to 0.10 mg/kg and the limit of detection is 0.001 mg/kg.

The HPLC methods described above appear suitable for regulatory use, although additional validation for appropriate species/matrix combinations is required. In addition, alternatives for highly chlorinated solvents, such as chloroform, may be required.

Choice of marker residue

Imidocarb is the choice as marker residue in all tissues, although the radiolabel study in cattle did reveal the presence in some samples of small amounts of metabolites that constituted <10% of the total radioactivity. This study was used to establish the ratio of total residue to marker residue. The proposed regulatory method includes an enzyme digestion step, not included in the parent compound analysis in the radiolabel depletion study, but results should be comparable when corrected for recovery.

Maximum Residue Limits

In recommending MRL's, the Committee took into account the following factors:

- An ADI of 0-10 µg per kg of body weight was established, which results in a maximum ADI of 600 µg for a 60-kg person.
- The ratios of parent compound to total residues determined in the radiolabel study were as follows: liver, 68%; kidney, 88%; muscle, 88%; and milk, 77%. Data were not available for fat, so a factor based on the lowest ratio in liver was applied.
- Imidocarb is the appropriate marker residue. Liver and muscle are the recommended target tissues.
- A suitable analytical method is available for analysis of imidocarb residues in edible tissues and milk.

On the basis of the above considerations, the Committee recommended the following temporary MRL's for edible tissues of cattle and cattle milk, expressed as parent drug:

Tissue	Recommended MRL (µg/kg)	Food Factor (g)	MR/TR	Consumption (µg)
Muscle	300	300	0.88	102
Liver	2000	100	0.68	294
Kidney	1500	50	0.88	85
Fat	50	50	0.68	4
Milk	50 (µg/L)	1500	0.77	97

The MRL's recommended above would result in a theoretical daily maximum intake of 582 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 L of milk.

The Committee requests that depletion studies be provided by 2001 in lactating and non-lactating cattle using the recommended SC dose of unlabeled imidocarb, and sample analysis using the proposed regulatory method with enzymatic digestion, before the temporary MRL's are considered for fully recommended MRL's. A depletion study is required in sheep, using the recommended dose and route of administration before MRL's can be considered for imidocarb as it is currently formulated.

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MOXIDECTIN

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ADDENDUM
to the moxidectin residue monographs prepared by the 45th, 47th and 48th meetings of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996, FAO Food and Nutrition Paper 41/9, Rome 1997 and FAO Food and Nutrition Paper 41/10, Rome 1998, respectively

INTRODUCTION

At the 45th meeting of the Committee in Geneva, 1995, additional data on the marker residues in deer tissue was requested. Data presented for review at this 1995 meeting clearly demonstrated that moxidectin is the marker residue in cattle and sheep tissue. It was not possible to make moxidectin the Marker Residue for deer tissues because the metabolism of moxidectin in deer was not known and therefore the relationship between moxidectin and the total residues was also unknown. The sponsor initiated an *in vitro* program to address these questions.

IN VITRO LIVER MICROSOME ASSAY STUDY WITH ^{14}C-MOXIDECTIN

Summary of Study

An assay was employed to describe and compare the metabolic profiles obtained for moxidectin in liver preparations from various animal species. The original submission contained similar data with this technique, which confirmed the metabolic profile of moxidectin in cattle tissue. Table 1 demonstrates that moxidectin is the main component of the extract following incubation, representing 70.25, 65.06 and 69.18 % of the recovered radioactivity in the microsomal preparations for cattle, sheep and deer, respectively. This suggests that moxidectin should be the marker residue in deer, as is the case in cattle and sheep tissue evaluated previously. The profile of the other peaks in the chromatogram is similar for these species with only minor differences observed among cattle, sheep and deer. For deer preparations, no metabolite represents greater than 10% of the total radioactivity. Individual chromatograms for all preparations are shown in Figure 1.

Comparative metabolism of moxidectin by deer hepatic microsomes (Done to GLP).

Livers of the different animals (deer, cow, sheep and goat) were collected from 4 individuals for each animal species. The microsomes were prepared by differential centrifugation and stored at –80°C until used. The microsomal preparations were validated using a comprehensive range of oxidative enzyme assays. After microsomal preparation and before use for *in vitro* metabolism study, total cytochrome P450 was determined by the method of Omura and Sato (1964). A complementary interspecies characterization of P450 isoenzymes was carried out by western blotting analysis of each microsomal preparation. Antibodies directed against the major hepatic P450 subfamilies (1A, 2B, 2C, 2E, 3A) were used (Towbin et al, 1979).

The optimal conditions of incubation were determined using cow liver microsomes. A test incubation mixture consisted of microsomal proteins (1 mg), 1 ml buffer (pH 7.4) and ^{14}C- moxidectin (10 µg, 500 µCi, >95% purity) dissolved in acetonitrile. All reactions were started by the addition of a NADPH-generating system and carried out at 37-38°C for either 30, 60, 90 or 120 minutes. The incubates were stored at –20°C before analysis.

The incubate was extracted by acetonitrile and purified by solid phase extraction. The methanol eluate was evaporated to dryness and the residual radioactivity taken up in methanol. One aliquot was used to check the recovery by liquid scintillation counting and another aliquot was used for HPLC analysis with a radioactive detector. The HPLC profiles are shown in Figure 1. The identification of metabolites was made by comparison with chromatographic profiles obtained in similar experiments by Zulalian *et al.* (1994).

The "*in vitro*" metabolism study showed that:

1. The microsomal enzyme activity measurements corresponded to the normal values reported for microsomal preparations obtained for these animal species;
2. The metabolic profiles obtained for all the species investigated showed that the metabolism of ^{14}C moxidectin is low. This observation is in good agreement with previous studies in this field (Zulalian *et al.*, 1994); and
3. Qualitatively the same metabolites were observed in all species, however interspecies differences appeared in the repartition between the different metabolites. Deer, rat and goat liver microsomes can be considered as lower metabolizers by comparison to sheep and cows preparations. The results are shown in Table 1.

Further experiments are in progress to confirm these preliminary results.

Figure 1. **HPLC radiochromatograms of microsomal incubates for different species**

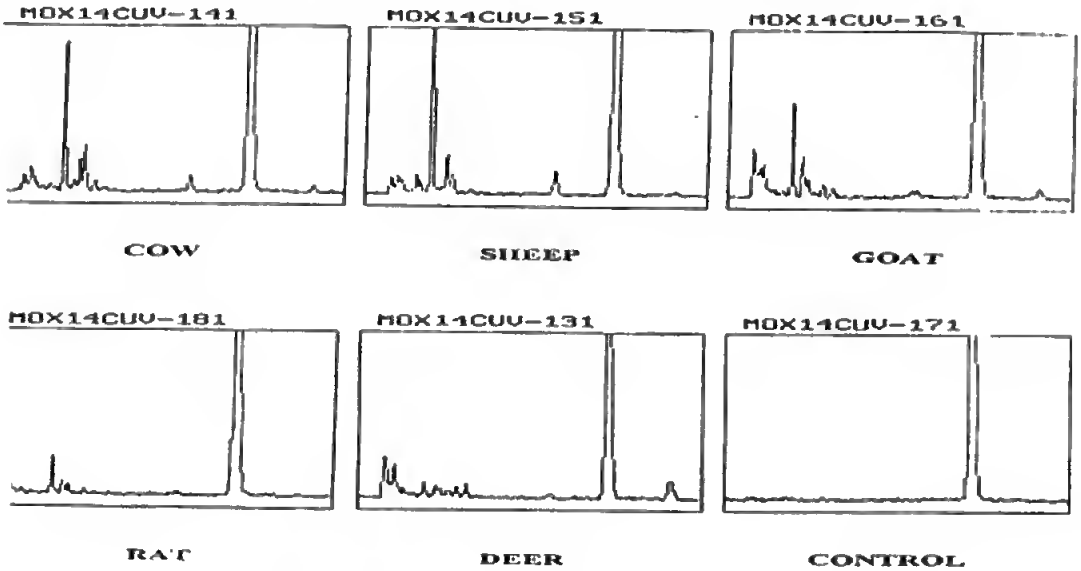

Table 1. **Compared percentages after HPLC of microsomal incubates following the incubation of microsomes with [^{14}C]-moxidectin.**

Peak Number	Retention Time (min)	Cow (%)	Sheep (%)	Goat (%)	Rat (%)	Deer (%)
1	3.52	1.84	1.69	6.11	1.12	9.34
2	4.73	3.16	2.65	2.29	0.73	4.83
3	5.85	0.24	0.10	0.21	0.19	0.38
4	6.18	0.39	1.10	0.07	0.24	0.09
5	7.41	0.10	0.72	0.31	0.12	0.60
6	8.61	13.12	21.25	5.15	3.07	1.77
7	9.92	0.61	0.21	2.70	1.19	1.61
8	10.72	2.11	2.84	1.01	0.80	0.85
9	11.51	3.72	1.61	--	0.06	1.08
10	12.70	1.09	0.22	0.85	0.51	1.53
11	14.00	0.48	0.21	0.74	0.18	2.12
12	24.62	2.01	2.78	0.90	0.94	1.24
13 (Moxidectin)	32.42	70.25	65.06	78.63	90.37	69.18
14	40.50	0.86	--	1.04	0.48	5.41

RESIDUE DEPLETION STUDY IN DEER.

Data for residues of unchanged moxidectin in red deer were presented at the 45th meeting of the Committee. Twenty red deer, 15-16 months old, were treated with moxidectin pour-on at a dose of 0.5 mg/kg BW. Five animals were sacrificed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the moxidectin content assayed. All residues were below the LOQ in muscle (<24 µg/kg), liver (<6 µg/kg) and kidney (<11 µg/kg). The residues in fat are shown in Table 2.

Table 2. **Residues (µg/kg) in fat of Red Deer after administration of a pour-on dose of 0.5 mg/kg BW**

Withdrawal time (days)	Mean conc. in fat	Calculated 99% upper CL
7	126	266
14	155	226
21	57	185
28	31	144

APPRAISAL

Moxidectin is a macrocyclic lactone antiparasitic drug that is used to control a number of internal and external parasites in sheep, cattle and deer.

Data presented at the 45th meeting of the Committee clearly demonstrated that moxidectin is the marker residue in cattle and sheep tissue. However, it was not possible to recommend MRLs for moxidectin in deer because the metabolism of moxidectin in deer was not known and the relationship between moxidectin and the total residues was also unknown. Additional data on the marker residues in deer tissue was requested. The sponsor reported on *in vitro* studies to address these questions.

A liver microsome assay with ^{14}C-moxidectin was employed to describe and compare the metabolic profiles obtained for moxidectin in liver preparations from various animal species. Livers of deer, cow, sheep and goat were collected from four individuals for each species and microsomes were prepared. The microsomes were incubated with ^{14}C-moxidectin at 37-38°C. The incubates were extracted and analyzed by HPLC. Results of the liver microsome studies indicate that moxidectin is the main component of the extract following incubation, representing 70%, 65% and 69% of the recovered radioactivity in the microsomal preparations for cattle, sheep and deer, respectively. The chromatographic profile of the other metabolites is similar for each species with only minor differences observed among cattle, sheep and deer. For deer preparations, no moxidectin metabolite represents greater than 10% of the total radioactivity. Results indicate that moxidectin metabolism in deer is comparable to cattle and sheep and that moxidectin should be the marker residue in all three species.

Residue data for moxidectin in red deer were presented in a study at the 45th meeting of the Committee. Twenty deer, 15-16 months old, were treated with moxidectin pour-on at a dose of 0.5 mg/kg BW. Groups of five animals were sacrificed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the moxidectin residues assayed. At all time points the residues were below the LOQ in muscle (<24 µg/kg), liver (<6 µg/kg) and kidney (<11 µg/kg) at each sampling time. The mean values (µg/kg) and the upper bound 99% CL (in parenthesis) in fat were at 7 days: 126 (266); 14 days, 155 (226); 21 days, 57 (185); and 28 days, 31 (144). These values are less than the proposed MRLs at all sampling times.

Maximum Residue Limits

The 45th meeting of the Committee established an ADI of 0-2 µg/kg, equivalent to 120 µg per day for a 60-kg person. The Committee recommended MRLs for cattle and sheep and provisional MRLs for deer of 500 µg/kg in fat, 100 µg/kg in liver, 20 µg/kg in muscle and 50 µg/kg for kidney expressed as parent drug based upon the following factors:

- Fat and liver are the target tissues;

- The marker compound is parent drug;
- 40% of the total residues in muscle, liver and kidney are unchanged drug;
- 75% of the total residues in fat are unchanged drug;
- Bound residues are 5-15% of the total residues and information is not available to discount them from the calculation of the MRL; and
- The LOQ of the analytical method is 10μg/kg.

The Committee recommends MRLs for deer as follows: 20 μg/kg in muscle; 100 μg/kg in liver; 50 μg/kg in kidney; and 500 μg/kg in fat expressed as parent drug.

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Zulalian, J., Stout, S.J., DaCunha, A.R., Garces, T. and Miller, P., (1994). Absorption, tissue distribution, metabolism and excretion of moxidectin in cattle. Journal of Agricultural and Food Chemistry, 42 (2), 388-392.

NICARBAZIN

First draft prepared by
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Australian Government Analytical Laboratories
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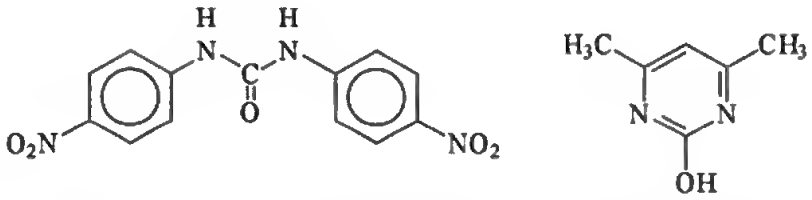
IDENTITY

Chemical name: N,N'-Bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone (equimolar complex).
4,4'-Dinitrocarbanilide and 4,6-dimethyl-2-pyrimidinol (equimolar complex).

Chemical structure:



Molecular formula: $C_{13}H_{10}N_4O_5$ (phenyl urea portion of the molecular complex)
$C_{19}H_{18}N_6O_6$ (phenyl urea – dimethylpyrimidinone, 1:1 molecular complex)

Molecular weight: 292.25 (phenyl urea portion of the molecular complex)
426.38 (phenyl urea – dimethylpyrimidinone, 1:1 molecular complex)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Purity: nicarbazin consists of 1:1-molar mixture of N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone with a purity of not less than 96%

Appearance: pale yellow powder

Decomposion point: 265-275°C (dec.)

Solubility (g/L):

water	almost insoluble, complex dissociates slowly
alcohols, ether and chloroform	very slightly soluble, complex dissociates slowly
dilute acids	almost insoluble, complex dissociates faster
dimethylformamide	soluble (1:700), with dissociation of complex
dimethylsulfoxide	soluble, with dissociation of complex

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Nicarbazin is a coccidiostatic drug used for the prevention of caecal and intestinal coccidiosis in broiler chickens. This is its sole use in animal or poultry production. The complex between N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone which constitutes the commercial drug appears to be essential for the observed coccidiostatic

properties which are not duplicated by an equimolar mixture of the individual constituents, N,N'-bis(4-nitrophenyl)urea plus 4,6-dimethyl-2(1H)-pyrimidinone.

Dosage

Nicarbazin is fed continuously, mixed with feed, at a rate of 125 mg/kg (0.0125%).

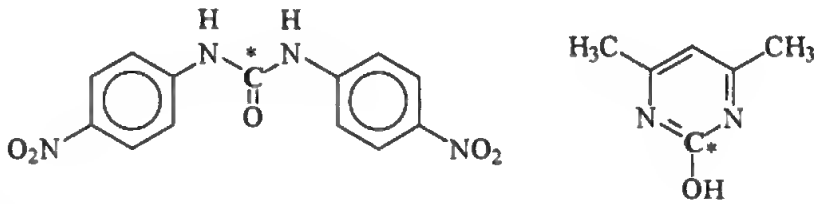
METABOLISM

Radiolabeled Nicarbazin

Various excretion and pharmacokinetic studies were conducted with 3 separate preparations of nicarbazin labeled with ^{14}C. Earlier studies utilisised two preparations where either the carbonyl of the bis-4-nitrophenyl urea was specifically ^{14}C-labeled or the 2 position of the pyrimidinone ring was specifically ^{14}C-labeled. The molecular labelling sites for these preparations are shown in Figure 1.

As nicarbazin passes into solution, the equimolar complex, comprising nitrophenylurea and pyrimidinone components, dissociates. Each of the two halves of the original complex is metabolised individually at separate rates (Porter and Gilfillan, 1955). The early labeling studies were therefore designed to determine the metabolic fate of both moieties comprising the original nicarbazin complex.

Figure 1 **Structures of ^{14}C-radiolabeled preparations of nicarbazin used in pharmacokinetic, metabolism and residue depletion studies**



Pharmacokinetics

Bioavailability and Excretion

Five studies in which chickens were dosed with ^{14}C-nicarbazin, radiolabeled either on the phenylurea or the pyrimidinone portion of the complex, are summarised in Tables 1 and 2. Birds were administered appropriately labeled ^{14}C-nicarbazin in the feed for 3 days followed by a withdrawal period of 4 days. During the whole period of the experiments, urine and faeces were collected and the radioactivity measured. Table 1 shows the recovery, during the course of the experiments, of ^{14}C from urine and faeces of chickens fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled in either the phenylurea or pyrimidinone portion of the drug for 3 days followed by a 4-day withdrawal period. Table 2 shows the results of other experiments where total radioactivity present in urine and faeces were measured over the 7-day period and compared with the administered doses (Nessel, 1977). Recovered radioactivity accounted for practically all of the administered ^{14}C-nicarbazin, averaging 94% in the chickens fed phenylurea-labeled nicarbazin and 104% in chickens fed pyrimidinone-labeled nicarbazin.

In separate experiments (Nessel, 1977), the distribution of ^{14}C nicarbazin in urine and faeces was measured in three different studies using the same labeled substances and the same feeding regimen as that used above (3 days on drug, 4 days withdrawal). After 3 days, an average of 50% of excreted radiolabeled phenylurea had been excreted in faeces and 5.6% in the urine. At the end of 4 days after withdrawal of drug, a further 41% was excreted in faeces and 3.3% in urine. By contrast, after 3 days, an average of 83% of excreted radiolabelled pyrimidinone had been excreted in urine and 7.4% in the faeces. At the end of 4 days after withdrawal of drug, a further 7% was excreted in urine and 2.6% in faeces.

The main excretion pathway for the pyrimidinone portion of the complex was in the urine (90%). This demonstrated that this moiety was well absorbed; it was also rapidly eliminated since by the third day, 83% of the dose had already

been eliminated. By contrast, the phenylurea portion of the nicarbazin complex was predominantly excreted through the faces (90%) at a slower rate than the pyrimidinone and the majority of the radioactivity was recovered in the first 3 days after withdrawal of medication. The observed urinary concentrations were only 5-10% of those of the pyrimidinone, indicating that the phenylurea portion was not rapidly eliminated by the kidney. Plasma levels of the phenylurea portion of nicarbazin were higher than those of the pyrimidinone portion and the plasma clearance value for the phenylurea portion was much lower than the pyrimidinone (Nessel, 1977).

Table 1. **Recovery of radioactivity from urine and faeces of chickens fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled in either the phenylurea or pyrimidinone portion of the drug for 3 days followed by a 4-day withdrawal period.**

Day	pyrimidinone ring ^{14}C-labeled				bis-4-nitrophenyl urea ^{14}C-labeled			
	drug dose (mg)	% total drug fed*	% drug excreted (urine)*	% drug excreted (faeces)*	drug dose (mg)	% total drug fed*	% drug excreted (urine)*	% drug excreted (faeces)*
1-fed with drug	27.5	37	21.7	1.3	17.5	22	0.4	4.6
2-fed with drug	28.8	75	53.8	4.1	34.4	65	5.9	23.1
3-fed with drug	17.5	100	83.9	6.7	28.1	100	7.5	44.5
1-no drug		100	90.4	8.2		100	8.4	63.8
2-no drug		100	90.7	9.0		100	8.8	79.4
3-no drug		100	90.7	9.3		100	9.0	83.7
4-no drug		100	90.8	9.8		100	9.0	85.4
Total pyrimidinone moiety excreted = 100.6%					Total urea moiety excreted = 94.4%			

* calculated as the % of the total drug administered over a 3 day period

Table 2. **Total recovery of ^{14}C, calculated as nicarbazin equivalents, from urine and faeces of chickens with artificial anus, fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled in either the phenylurea or pyrimidone moiety for 3 days followed by a 4-day withdrawal.**

Day	pyrimidinone ring ^{14}C-labeled				bis-(4-nitrophenyl)urea ^{14}C-labeled			
	drug dose (mg)	% ^{14}C excreted (urine)*	% ^{14}C excreted (faeces)*	% drug excreted (total)#	drug dose (mg)	% ^{14}C excreted (urine)*	% ^{14}C excreted (faeces)	% drug excreted (total)#
7 week chickens	53.1	89.8	10.2	110	50	10.1	89.9	95.4
14 week chickens Experiment 1	NM	NM	NM	NM	78.8	7.1	92.9	93.1
14 week chickens Experiment 2	73.8	90.3	9.7	100.5	80	9.5	90.5	94.4
Mean Recovery (%)		90	10	105.3		8.9	91.1	94.1

* calculated as the % of the combined total ^{14}C-drug excreted in urine and faeces
\# calculated as the % of the administered ^{14}C-drug excreted in urine and faeces combined

Metabolism

The pyrimidinone portion of nicarbazin is shown, in ^{14}C-studies, to be rapidly eliminated, with no discernible residues evident 4 days after drug withdrawal. No metabolism studies have been conducted for this residue because of the very

low potential for detrimental residues with this molecule. By contrast, the phenylurea portion of nicarbazin is excreted much more slowly and leads to significant residues in liver and kidney.

Chickens were fed a diet containing 125 mg/kg of nicarbazin for 7 days and successively sacrificed between day 2 and day 7. Both urea and pyrimidinone moieties were ^{14}C-labeled as shown in Figure 1 and results of this study are summarised in Table 3. ^{14}C-Pyrimidinone-labeled concentrations peaked at 2.1 mg/kg in the plasma on day 2 whereas maximum ^{14}C-phenylurea-labeled plasma concentrations of 3.8 mg/kg occurred on day 4. Concentrations of the ^{14}C-labeled urea portion of the complex were much higher in liver and kidney than in plasma and muscle whereas, although ^{14}C-labeled pyrimidinone concentrations are highest in kidney, they are comparable in all four matrices. Liver and kidney concentrations of the pyrimidinone portion of the nicarbazin complex are about 10 times less than the concentrations of the phenylurea portion of the complex (Nessel, 1977).

Early Merck radiolabel studies conducted in the 1950s only positively identified or quantified, albeit colourimetrically, one metabolite, N,N'-bis(4-acetylaminophenyl)urea. From colourimetric analysis it was concluded that the ^{14}C-radiolabeled phenylurea was not extensively metabolised and was almost completely excreted 4 days after withdrawal of medication.

Table 3. Tissue profiles between days 2-7 in plasma, liver, kidney and muscle of 4-week old chickens fed for seven days a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled both in the phenylurea and pyrimidinone portions of the complex.

Day of Sacrifice	Concentration, calculated as nicarbazin, (mg/kg)*							
	Plasma		Liver		Kidney		Muscle	
	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr
Day 2	2.50	2.07	23.11	2.36	18.26	3.52	4.11	2.13
Day 3	2.54	1.84	26.48	2.15	19.26	3.09	3.86	2.03
Day 4	3.80	1.58	34.79	1.89	27.44	2.48	5.57	1.52
Day 5	2.75	1.07	29. 82	1.32	20.35	1.96	4.52	1.42
Day 7	3.33	1.79	33.78	2.08	26.74	2.95	5.98	1.63

^{14}C urea = carbonyl of the bis-(4-nitrophenyl)urea specifically ^{14}C-labeled
^{14}C pyr = 2-position of the pyrimidinone ring specifically ^{14}C-labeled; * mean of two replicates

A concern in early work based on radiolabeling studies lies in the placement of the ^{14}C-atom at the carbonyl group of the ^{14}C-radiolabeled phenylurea. This position would be expected to be labile and therefore the radiolabel is likely to be lost at an early stage of a possibly extensive metabolic degradation of the phenylurea portion of nicarbazin. A metabolite study using nicarbazin, generally ^{14}C-radiolabeled in the phenylurea portion, has been conducted (Manthey, 1986). Hubbard x White Mountain broiler chickens, approximately 6 weeks old, were fed 50 mg/kg ^{14}C-nicarbazin, alone or with ionophore, for 5 days and killed immediately at end of drug administration. The metabolic pattern was the same with or without accompanying ionophore. Parent nicarbazin accounted for about 79% of total liver radioactivity with about 10% of metabolite M-3 and 2% of metabolite M-1. Kidney radioactivity was 6% parent and 13% metabolite M-1 with the remainder as non-extractable polar activity. Metabolite M-2 was only found in excreta. The mean net radioactivity, calculated as mg/kg nicarbazin in tissues of chickens dosed with ^{14}C-nicarbazin in the above study is shown in Table 4.

Table 4. Drug-metabolite profiles in liver, kidney, muscle, skin and fat of chickens given 50 mg/kg of radiolabeled ^{14}C-nicarbazin for 5 days.

Study No.	No of Chickens	Tissue concentration, calculated as nicarbazin, (mg/kg)				
		Liver	Kidney	Muscle	Skin	Fat
1	6	10.84	7.17	1.47	1.52	1.77
2	8	11.64	7.57	1.35	1.62	2.00
3*	8	14.00	10.09	2.13	2.26	2.65

* = nicarbazin fed together with an ionophore

Metabolites of Nicarbazin

Metabolite Identification Code	Identity
M1	N,N'-bis(4-acetylaminophenyl)urea
M3	N,N'-4-acetylamino-4'-nitrodiphenylurea
M2	1,4-diacetylaminobenzene

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Tissue distribution and elimination studies carried out over a number of years by Merck & Co. and by Eli Lilly & Co. have been summarised as a consolidated document for submission to EU-SCAN for final compound evaluation (Merck and Lilly, 1986). Because of the non-availability of most of the source documents on which this summary was based, only the results presented in that summary paper are discussed here.

Results of studies in which chickens were fed a diet containing 125 mg/kg of nicarbazin for 7 days and successively sacrificed between day 2 and day 7 have been discussed earlier and are summarised in Table 3.

Residue depletion studies in which chickens were fed 125 mg/kg nicarbazin labeled in both moieties (see Figure 1) for 3 days are shown in Table 5 (Merck and Lilly, 1986). The birds were sacrificed successively, commencing at the withdrawal of medication (day 3), then after two days post-withdrawal (day 5) and then at three day intervals thereafter until day 14 and, finally, at day 21.

The data contained in Table 5 shows the rapid elimination of both drug and metabolites from the birds. Based on an assay sensitivity of 0.003-0.004 mg/kg, all tissues were essentially devoid of ^{14}C-residues from the pyrimidinone portion of nicarbazin by the fifth day after withdrawal. ^{14}C-Residues emanating from the phenylurea portion of nicarbazin were essentially only present in liver 5 days after withdrawal.

Table 5. **Tissue profiles in plasma, liver, kidney and muscle of chickens fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled both in the phenylurea and pyrimidinone portions of the complex for 3 days followed by withdrawal of medication.**

Day of Sacrifice	Concentration, calculated as nicarbazin, (mg/kg)*							
	Plasma		Liver		Kidney		Muscle	
	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr
Day 3*	4.48-5.32	1.50-1.79	41.48-51.5	1.80-2.38	36.58-40.05	2.63-3.73	8.15-9.30	1.78-2.00
Day 5#	<0.04	<0.04	0.2-0.34	0-0.216	0-0.085	<0.04	<0.04	0-0.18
Day 8#	<0.04	<0.04	0.105-0.228	<0.04	0-0.13	<0.04	<0.04	0-0.115
Day 11*	<0.04	<0.04	0.080-0.088	<0.04	<0.04	<0.04	<0.04	<0.04
Day 14*	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Day 21*	<0.04	<0.04	0.053-0.073	<0.04	<0.04	<0.04	<<0.04	<0.04

^{14}C urea = carbonyl of the bis(4-nitrophenyl)urea specifically ^{14}C-labeled
^{14}C pyr = 2-position of the pyrimidinone ring specifically ^{14}C-labeled
* range of values from two birds; # range of values from five birds

In studies conducted by Lilly (Merck and Lilly, 1986), chickens were fed either 50 or 60 mg/kg nicarbazin, ^{14}C-radiolabeled on either the urea or pyrimidinone portion of the molecular complex, in combination with ionophores. The chickens were dosed for 5 days and killed immediately after the final dose. Results, shown in Table 6, illustrate a tissue distribution pattern in line with other studies discussed earlier. The pyrimidinone portion of the complex contributes much lower residues, at the time of sacrifice, than do the dinitrophenylurea residues. As with all other studies, the dinitrophenylurea residues are highest in liver and kidney.

Table 6. **Drug residue profiles in liver, kidney, muscle, skin and fat of chickens administered radiolabeled ^{14}C-nicarbazin for 5 days and sacrificed immediately.**

Study No.	Labeled Portion and Dose	Tissue concentration, calculated as nicarbazin, (mg/kg)				
		Liver	Kidney	Muscle	Skin	Fat
1	^{14}C-urea, 60 mg/kg	14.86	11.46	2.36	2.59	2.43
1	^{14}C-pyrimidone, 60 mg/kg	0.28	0.34	0.31	0.18	-
2	^{14}C-urea, 50 mg/kg	11.15	7.24	1.18	1.81	1.93

A residue depletion study has also been conducted in which nicarbazin, ^{14}C-labeled in the phenylurea portion of the molecule, was fed to chickens in combination, with an ionophore, at 50 mg/kg for six days. Groups of four birds were sacrificed at 0, 1, 3, 5 and 7 days after withdrawal of drug. Total radioactivity was monitored and the concentration of the phenylurea portion of the drug was determined by HPLC. Results of these determinations are shown in Table 7. The results from radioactive and HPLC determinations were in good agreement for muscle , fat and skin, indicating that metabolites were not present in these tissues. At day 5 after drug withdrawal, liver was the only tissue with significant residues of parent drug.

Table 7. **Residue profiles in liver, kidney, muscle, skin and fat of chickens given 50 mg/kg BW of ^{14}C-nicarbazin for 6 days and sacrificed at various times after withdrawal of drug.**

Tissue		Sacrifice Day after withdrawal of Drug				
		0	1	3	5	7
Liver	NC(mg/kg)	10.24	4.82	0.50	0.10	ND
	TR (mg/kg)	16.81	7.88	1.19	0.22	0.06
	NC/TR ratio	0.61	0.61	0.42	0.45	-
Kidney	NC(mg/kg)	2.95	1.32	0.1	ND	NA
	TR (mg/kg)	12.09	5.38	0.8	0.14	0.03
	NC/TR ratio	0.24	0.25	0.13	-	-
Muscle	NC(mg/kg)	1.52	0.49	0.1	ND	NA
	TR (mg/kg)	2.19	0.76	0.11	0.02	ND
	NC/TR ratio	0.69	0.64	0.91	-	-
Skin	NC(mg/kg)	2.98	1.09	0.1	ND	NA
	TR (mg/kg)	2.44	0.85	0.13	0.03	0.01
	NC/TR ratio	1.22	1.28	0.77	-	-
Fat	NC(mg/kg)	2.67	0.78	0.12	ND	NA
	TR (mg/kg)	2.85	0.97	0.13	0.02	0.01
	NC/TR ratio	0.94	0.80	0.92	-	-

NC = N,N'-bis(4-nitrophenyl)urea; TR = Total residues; ND = not detected; NA = not analysed

The ratio of nicarbazin residues, determined by HPLC, to total residues, determined radiometrically for withdrawal days 0, 1, 3 and 5 were: in liver 0.61, 0.61, 0.42 and 0.45, respectively, in kidney 0.24, 0.25, 0.13 and not measurable, respectively, in muscle = 0.69, 0.64, 0.91 and not measurable, respectively, and in skin and fat, all values measured lay between 0.77 and 1.28.

Residue Depletion Studies Using Unlabeled Nicarbazin

A residue depletion study was conducted in which chickens were fed a diet containing 125 mg/kg nicarbazin from 3 days of age until suspension of medication at 44 days of age. Groups of 8 birds (4 male and 4 female) were sacrificed at

1, 3, 5, 7 and 9 days after the final dose. Edible tissues were analysed for the phenylurea portion of nicarbazin by a pulse polarographic method with a limit of quantification of 0.1 mg/kg and a limit of detection of 0.03 mg/kg (Wood and Dowling, 1980). The results of this study are shown in Table 8. These data indicated that the highest residue concentration occurred in liver at all withdrawal times, followed by kidney, skin/fat and muscle, respectively. Kidney, skin/fat and muscle residue values fell to < 0.1 mg/kg in four to six days and were about ten times lower than liver residue values at comparable withdrawal times after day 1. There was no evidence of drug recycling by chickens picking at the litter of the holding pen. This study also found that residues in frozen tissue were stable for at least five months at -20^{0}C. Analytical results are not corrected for recoveries, which exceeded 80%.

Table 8. **Drug residues in liver, kidney, muscle and skin/fat of chickens given 125 mg/kg of nicarbazin for 42 days and sacrificed at various times after withdrawal of drug.**

Day of sacrifice after withdrawal	Tissue concentration ranges* of nicarbazin, determined as phenylurea (mg/kg)			
	Liver	Kidney	Muscle	Skin/Fat
Day 1	14.4-21.0	2.8-5.4	1.4-2.2	1.6-3.0
Day 3	3.0-9.4	0.18-2.5	0.12-0.78	0.18-0.86
Day 5	0.40-2.7	<0.1-0.28	<0.1-0.1	<0.1-0.22
Day 7	0.14-0.59	<0.1	<0.1	<0.1-0.1
Day 9	<0.1-0.12	<0.1	<0.1	<0.1

LOQ = 0.1 mg/kg; LOD = 0.03 mg/kg; *8 birds sacrificed at each time point

In a more recent study (Kramer, 1990), chickens were dosed 125 mg/kg of nicarbazin in the feed for 49 days. After withdrawal of drug, groups of 4 birds (2 male, 2 female) were sacrificed at 24, 36, 48, 60 and 72 hours. N,N'-Bis-(4-nitrophenyl)urea residue concentrations in liver, muscle and skin/fat were determined by the HPLC method of Lewis (1989). The results, shown in Table 9, were in line with the earlier study but were not taken beyond 3 days withdrawal. At that time, muscle and skin/fat residues were at or below 0.2 mg/kg while the highest liver residue concentration measured was 3.39 mg/kg.

Table 9. **Drug residues in liver, muscle and skin/fat of chickens given 125 mg/kg of nicarbazin for 49 days and sacrificed at various times after withdrawal of drug.**

Hour of sacrifice after withdrawal	Tissue concentration ranges* of nicarbazin, determined as phenylurea (mg/kg)		
	Liver	Muscle	Skin/Fat
Hour 24	2.69-9.12	0.85-1.23	0.66-0.99
Hour 36	2.79-7.09	0.37-0.88	0.68-1.06
Hour 48	3.33-4.79	0.23-0.45	0.43-0.66
Hour 60	2.71-3.42	<0.1-0.233	0.14-0.51
Hour 72	0.90-3.39	<0.1-0.21	<0.1-0.28

* four birds sacrificed at each time point (2 male, 2 female)

METHODS OF ANALYSIS IN CHICKEN TISSUES AND EGGS

Earlier reported methods for the analysis of nicarbazin were based on either differential pulse polarography or colourimetry. These lack the necessary sensitivity or selectivity of a modern regulatory method but were used, none the less, to accumulate some of the residue data discussed above (eg. Michielli and Downing 1974). Residues in chicken tissues, down to the 1 mg/kg level generally required by regulatory agencies, has also been achieved by pulse polarography. The 4,4'-dinitrophenylurea portion of the complex was extracted with ethyl acetate. After removal of solvent, kidney and liver samples were cleaned up by a series of hexane washes of acetonitrile and acetonitrile/water solutions containing a small amount of dimethylsulfoxide (DMSO), followed by extraction into dichloromethane. After removal of dichloromethane, a pulse polarogram was obtained on a DMSO solution of the residue after washing with hexane/toluene. The resulting polarograms were essentially clean for tissues from untreated chickens, and recoveries of

fortified tissues at the 0.1-0.4 mg/kg level averaged 73%, 76%, 85% and 94% for liver, kidney, muscle and skin-fat, respectively (Wood and Downing, 1980). The limit of quantification in tissues was 0.1 mg/kg, but the estimated limit of detection was much lower at 0.03 mg/kg.

The first liquid chromatographic (HPLC) method for the determination of nicarbazin residues in chicken tissue appeared in 1983 (Takahashi and Yoshida) and was followed by a HPLC procedure for the determination of the phenylurea portion of nicarbazin in eggs, using UV-spectrophotometry as a confirmatory tool (Malisch, 1986).

A recent method for the analysis of the phenylurea portion of nicarbazin employed LC determination, with UV-detection, followed by LC-thermospray mass spectrometric confirmation of nicarbazin in chicken tissues (Lewis *et al.*, 1989). The dinitrophenylurea portion of nicarbazin was extracted from tissues with ethyl acetate. After filtration and evaporation, the extract was purified by liquid-liquid partitioning with acetonitrile-hexane followed by alumina chromatography. The dinitrophenylurea was separated and measured by reverse-phase LC on an octadecylsilyl column with UV-detection at 340 nm. The overall average recovery of the phenylurea from fortified tissues was 83.4±3.1% with coefficients of variation (CVs) below 10%. The lowest level validated in liver, kidney, muscle and fat tissues by this procedure was 0.10 mg/kg. The limit of detection was estimated to be 0.020 mg/kg. The identity of the analyte was confirmed by subjecting the purified extracts to LC with thermospray-mass spectrometric analysis using negative-ion detection and selective ion monitoring. Three ions at m/z 302 (M^-), 272 and 164 are characteristic of the analyte. A validation study of the method by the US-FDA has been reported using chicken liver and muscle at 2, 4 and 8 mg/kg using four laboratories (Leadbetter and Matusik, 1993). At the 4 mg/kg level, mean laboratory recoveries and CVs were 87.1% (10.9%) and 87.4% (7.5%) in muscle and liver, respectively (n=21). A separate set of validation data was generated for this method during a 1990 residue depletion study discussed earlier (Hazelton - Planalquimica, 1990). A similar LC method, based on a solid phase dispersion clean up has also been published (Schenck, 1992) but offers no obvious advantage over the Lewis method.

Although nicarbazin is not approved for use in laying hens, several methods are available that can monitor accidental residues in eggs, exemplified by a recent LC method (Kondo *et al.*, 1993). The recovery of nicarbazin added to eggs was 90.2% and the detection limit was 0.005 mg/kg. Nicarbazin was detected in 10% of eggs obtained by feeding chickens with a diet contaminated with nicarbazin within the range 0.07 to 1.39 mg/kg, but was not detected in eggs obtained commercially.

APPRAISAL

Nicarbazin, which has had a long history of use (four decades), is a coccidiostatic drug as an aid for the prevention of faecal and intestinal coccidiosis in broiler chickens. The complex between N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone which constitutes the commercial drug appears to be essential for the observed coccidiostatic properties. Nicarbazin is fed continuously, mixed in starter rations at a rate of 125 mg/kg (0.0125%).

Pharmacokinetics

An excretion study was performed in chickens using nicarbazin, [^{14}C]-radiolabeled in both phenylurea and pyrimidinone portions of the molecule. The main excretion pathway for the pyrimidinone portion of the complex was in the urine (>90%). This demonstrated that this moiety was well absorbed. It was also rapidly eliminated and by the third day after the last dose, 83% of the pyrimidinone had been eliminated. By contrast, the phenylurea portion of the nicarbazin complex was predominantly excreted (90%) through the faeces and at a slower rate than the pyrimidinone but the majority of the radioactivity was recovered in the first 3 days after withdrawal of medication. The observed urinary concentrations for the phenylurea portion were only 5-10% of those of the pyrimidinone indicating that kidney was not the major elimination pathway.

Metabolism

Broiler chickens were fed a diet containing 125 mg/kg of nicarbazin, [^{14}C]-labeled in both phenylurea and pyrimidinone, for 7 days and groups of birds were sacrificed between day 2 and day 7. Concentrations of the [^{14}C]-labeled phenylurea portion of the complex were much higher in liver and kidney than in plasma and muscle. [^{14}C]-labeled pyrimidinone concentrations were highest in kidney, they were not significantly lower in muscle, plasma and liver. Liver and kidney concentrations of the pyrimidinone portion of the nicarbazin complex are about 10 times less than the concentrations of the phenylurea portion of the complex. The rapid elimination of the pyrimidinone portion of nicarbazin and the non-detection of metabolites has led to an almost exclusive focus on the phenylurea portion of the complex in subsequent metabolism and residue depletion studies.

In another study, broiler chickens were fed 50 mg/kg [^{14}C]-nicarbazin, alone or with an ionophore, for 5 days and sacrificed immediately at end of drug administration. The metabolic pattern observed was the same with or without accompanying ionophore. The phenylurea portion of the parent nicarbazin accounted for about 79% of total liver radioactivity with about 10% of metabolite M-3 (N,N'-4-acetylamino-4'-nitrodiphenylurea) and 2% of metabolite M-1 [N,N'-bis(4-acetylaminophenyl)urea]. Kidney radioactivity comprised 6% of parent and 13% of metabolite M-1 with the remainder as non-extractable residues. From these data, N,N'-bis(4-nitrophenyl)urea was selected as the marker residue in all residue depletion studies.

Residue Depletion Studies

Residue depletion studies in which chickens were fed 125 mg/kg nicarbazin for 3 days with [^{14}C]-radiolabel in both moieties, showed the rapid elimination of both parent drug and metabolites from the birds. Based on an assay sensitivity of 0.003-0.004 mg/kg, all tissues were essentially devoid of radiolabeled residues from the pyrimidinone portion of nicarbazin by day five after withdrawal. [^{14}C] residues emanating from the phenylurea portion of nicarbazin were only present in liver five days after withdrawal.

In a second study, chickens were fed either 50 or 60 mg/kg nicarbazin, with a ^{14}C-radiolabel on either the phenylurea or pyrimidinone portion of the molecular complex, in combination with ionophores. The chickens were dosed for 5 days and sacrificed immediately after the final dose. The pyrimidinone portion of the complex contributed much lower residues than did the dinitrophenylurea residues. The ratios of phenylurea to pyrimidinone residues, at the time of sacrifice, were 53:1, 34:1, 8:1 and 14:1 in liver, kidney, muscle and fat, respectively. As with all other studies, the dinitrophenylurea residues were highest in liver and kidney.

Another residue depletion study was conducted in which nicarbazin was fed to chickens for six days at 50 mg/kg using ^{14}C-label in the phenylurea portion of the molecule, in combination with an ionophore. Total radioactivity was monitored and the concentration of the phenylurea portion of the drug was determined by HPLC. Table 10 shows the results of this study and also shows the ratios of N,N'-bis(4-nitrophenyl)urea, the marker residue, to the total residues.

Table 10. Residues of nicarbazio in chickens in mg/kg fed nicarbazin at 50 mg/kg BW for 6 days

Tissue	Portion of Nicarbazin	Sacrifice Day after withdrawal of Drug				
		0	1	3	5	7
Liver	NP	10.24	4.82	0.50	0.10	ND
	TR	16.81	7.88	1.19	0.22	0.06
	NP/TR	0.61	0.61	0.42	0.45	-
Kidney	NP	2.95	1.32	0.1	ND	NA
	TR	12.09	5.38	0.8	0.14	0.03
	NP/TR	0.24	0.25	0.13	-	-
Muscle	NP	1.52	0.49	0.1	ND	NA
	TR	2.19	0.76	0.11	0.03	ND
	NP/TR	0.69	0.64	0.91	-	-
Skin	NP	2.98	1.09	0.1	ND	NA
	TR	2.44	0.85	0.13	0.03	0.01
	NP/TR	1.22	1.28	0.77	-	-
Fat	NP	2.67	0.78	0.12	ND	NA
	TR	2.85	0.97	0.13	0.02	0.01
	NP/TR	0.94	0.80	0.92	-	-

NP = N,N'-bis(4-nitrophenyl)urea; TR = Total residues; ND = not detected; NA = not analyzed.

In one study, chickens were dosed 125 mg/kg of nicarbazin daily in the feed for 49 days. After withdrawal of drug, birds were sacrificed at 24, 36, 48, 60 and 72 hours. Residue concentrations in liver, muscle and skin/fat were determined by HPLC. At 72 hours after withdrawal, muscle and skin/fat residues were at or below 0.2 mg/kg. The

highest liver residue concentration measured was 7.09 mg/kg 36 hours following withdrawal. In an earlier, long term feeding study, young chicks were fed a diet containing 125 mg/kg nicarbazin daily from 3 days of age until 44 days of age. Groups of birds were sacrificed at 1, 3, 5, 7 and 9 days after the final dose. The highest residue concentration of N,N'-bis-(4-nitrophenyl)urea occurred in liver at all withdrawal times. Residues were lower in kidney, skin/fat and muscle, respectively. Kidney, skin/fat and muscle residue values declined to less than 0.2 mg/kg at five days and were about ten times lower than liver residue values at all withdrawal times after day 1. Marker residue concentrations in liver ranged from 14.4-21 mg/kg at day 1, 3.0-9.4 mg/kg at day 3, 0.4-2.7 mg/kg at day 5, 0.14-0.59 mg/kg at day 7, and <0.1-0.12 mg/kg at day 9.

Methods of Analysis

Several HPLC procedures for the determination of residues of the phenylurea portion of nicarbazin in chicken tissue are available. These methods, which employ UV-detection, appear to be suitable for the routine monitoring of nicarbazin residues. A limit of detection down to 0.02 mg/kg can be acheived. A recent method for the analysis of the phenylurea portion of nicarbazin employed HPLC determination, with UV-detection, followed by LC-thermospray mass spectrometric confirmation of nicarbazin in chicken tissues. The overall average recovery of the phenylurea from fortified tissues was 83% with coefficients of variation below 10%. An analytical method validated by six laboratories in a trial, organised by the US-FDA, has a limit of quantification of 0.1 mg/kg and is suitable for routine monitoring of an MRL of 0.2 mg/kg in all tissues. Although nicarbazin is not approved for use in laying birds, suitable methods are available which can monitor residues in eggs derived from accidental contamination with a detection limit of 0.005 mg/kg.

Maximum Residue Limits

Based on the ADI of 0-400 µg/kg established by the Committee, the permitted daily intake of parent drug and/or its equivalents is 24000 µg for a 60-kg person. In recommending MRLs for nicarbazin in broiler chickens, the Committee took the following factors into consideration:

- The limit of quantification of the analytical method is 0.1 mg/kg for all tissues.
- Nicarbazin is for use in broiler chickens only during the first 28 days post hatching.
- The marker residue is N,N'-bis-(4-nitrophenyl)urea.
- Mean ratios of marker residue to total residues in liver, kidney, muscle and skin/fat are approximately 0.45, 0.25, 0.65, and 0.90, respectively.
- The recommended MRLs are consistent with good practice in the use of veterinary drugs.

The Committee recommends MRLs of 200 µg/kg for muscle, liver, kidney and fat/skin in broiler chickens as N,N'-bis-(4-nitrophenyl)urea. Using these MRLs and food consumption factors of 300 g muscle, 100 g liver, 50 g kidney and 50 g fat, the theoretical maximum daily intake of residues as nicarbazin equivalents is 187 µg.

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PROCAINE BENZYLPENICILLIN

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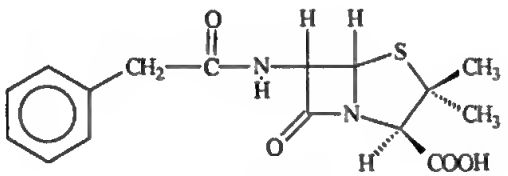
IDENTITY

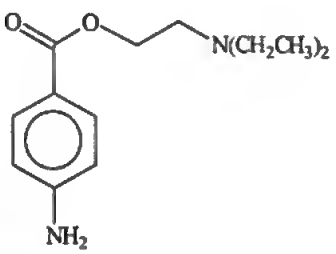
Chemleal name: [2S-(2α,5 α,6 β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-aza-bicyelo-[3.2.0]heptane-2-carboxylie aeid compounded with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate

Synonyms: benzylpenicillin, benzylpenicillin procaine, procaine penieillin G, Abbocillin-DG, Afsillin, Ampin-penicillin, Aquacillin, Aquasuspen; Avloprocil, Cilicaine, Crysticillin, Despacilina, Despocillin, Distaquaine, Dorsallin "A.R.", Duracillin, Flo-Cillin Aqueous, Hydracillin, Ilcocillin P, Kabipenin, Ledercillin, Lentieillin, Mamunaeillin, Megapen, Mylipen, Neoproc, Penaquacaine G, Pen-Fifity, Premocillin, Procanodia, Pro-Pen, Wycillin.

Struetural formula:



Penicillin G



Proeaine

Molecular formula: $C_{29}H_{38}N_4O_6S\text{-}H_2O$

Molecular weight: 588.73

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active Ingredient: procaine benzylpenicillin (penicillin potency approx. 1000 units/mg)

Appearance: monoclinic hemimorplue erystals from methanol-water

Melting point: 106-110°C (with decomposition)

Solubility: Soluble in water, methanol and isopropanol; moderately soluble in ethyl acetate and toluene; slightly soluble in benzene, petroleum ether and carbon tetraehloride; insoluble in iso-octane.

Optleal rotation: dextrorotary in aqueous solutions.

Ultravlolet maxima: 190 nm at pH 3.4

Stability: procaine benzylpenicillin is rapidly inactivated by acids, alkalis and oxidizing agents.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Procaine benzylpenicillin, a combination of two compounds, benzylpenicillin (penicillin G) and procaine (1:1), is one of a number of benzylpenicillin compounds that is widely used in many countries for the treatment and prevention of bacterial infections in cattle, horses, swine, sheep, chickens and turkeys, as well as in various minor species such as rabbits, quail and pheasants. It is available in both injectable and feed additive formulations, frequently in combination with other antimicrobial compounds. Benzylpenicillin is active against gram-positive bacteria, while procaine, one of the first useful local anaesthetics, has been used as such for almost a century.

Benzylpenicillin procaine is hydrolyzed in the muscle to benzylpenicillin and procaine, with subsequent absorption of benzylpenicillin from the muscle. The absorption of procaine is very slow. Usage of procaine benzylpenicillin is based on the prolongation of administration intervals due to the slow absorption of the drug from the injection site.

As procaine benzylpenicillin is poorly soluble in water (4 g/L), the usual formulation for administration is an injectable aluminium monostearate oily suspension. The particle size of the procaine benzylpenicillin in the suspension has considerable effect on the absorption rate of the drug from the injection site. Benzylpenicillin was last reviewed at the 36th Meeting of the Committee.

Dosage

A typical recommended dose by intramuscular injection (IM) in cattle, horses, sheep and swine of a 300,000 unit/mL formulation is 6,600 units/kg BW. As a feed additive, a typical dosage for poultry or swine is 55 mg/kg in the diet. Intramammary treatment is typically by administration of 100,000 units per quarter (Sundlof *et al*, 1988). In the studies reported, 1 mg procaine benzylpenicillin is equivalent to 1667 IU (international units).

METABOLISM

Pharmacokinetics

Toxicological Test Species

No studies involving laboratory animals were reviewed.

Metabolism in Food Animals

Cattle

Five calves (80-110 kg BW) received procaine benzylpenicillin as a single IM injection at a dose of 17760 ± 2325 IU/kg BW (Volner *et al*, 1991). A C_{max} of 2.1 ± 0.6 IU/mL was observed, with an elimination half-life ($t_{½}$) of 4.3 h and AUC of 18.25 ± 4.5 IU/mL/h. Co-administration of phenylbutazone at 24 mg/kg BW increased these parameters by about 20%. Decline in concentrations of benzylpenicillin in plasma was uniphasic.

In six cattle which received procaine benzylpenicillin IM at 10,000 IU/kg BW, in combination with dihydro-streptomycin (12.5 mg/kg BW), the absorption half-life for benzylpenicillin in serum was 9.96 ± 3.84 min., while the elimination half-life ($t_{½}$) was 1.87 ± 0.46 h. (Landoni and Errecalde, 1991). In another study (Bengtsson *et al.*, 1991), six calves (102 - 120 kg BW) received procaine benzylpenicillin at 30 mg/kg BW by IM injection in the neck. Benzylpenicillin administered in this experiment had a $t_{½}$ of 2.98 ± 1.20 h in serum and 10.21 ± 3.45 h in tissue cage fluid. Tissue cages are made from silastic rubber tubing and are implanted subcutaneously to provide a model for the distribution of antibacterial drugs in abscesses. C_{max} , the maximum concentration, was 5.37 ± 2.28 mg/L in serum and 1.52 ± 0.31 mg/L in tissue cage fluid, while the time to maximum concentration, T_{max}, was 1.5 ± 0.78 h in serum and 7.7 ± 2.22 h in tissue cage fluid.

Procaine benzylpenicillin was administered by IM injection to six mature cows at 20,000 IU/kg BW (Conlon *et al.*, 1993). The same 6 cows were administered with the drug 7 days later at the same dose by subcutaneous (SC) injection, repeated on 3 successive days. Blood samples were collected at 3, 5, 10, 15, 20, 30, 45, 60 and 90 min, and at 2, 4, 6, 8, 10, 12 and 24 h

following injection. Peak serum concentration appeared at 30 min after IM administration and at 2 h after SC injection, with a more rapid decline in plasma concentration of benzylpenicillin seen in the first 24 h following SC injection, compared to IM injection. Residues in tissues of the animals which were killed 5 days after the final SC injection were: liver, 1.00 ± 0.80 mg/kg; kidney (renal cortex), 0.90 ± 0.58 mg/kg; kidney (renal medula), 0.58 ± 0.17 mg/kg; muscle (diaphragm), 0.13 ± 0.11 mg/kg; muscle (gluteal), 0.10 ± 0.08 mg/kg; fat, 0.06 ± 0.04 mg/kg; muscle adjacent to injection site, 1.15 ± 1.27 mg/kg.

Two groups of 3 feedlot steers each received once daily on 5 successive days an injection of procaine benzylpenicillin IM of 24,000 or 66,000 IU/kg BW, respectively, with final injection being in the gluteal muscle, while a third group of three animals received a single injection of 66,000 IU/kg BW, IM in the neck, and a fourth group (3 animals) received the same dosage SC (Papich *et al*, 1993). The approved dose is 6,600 IU/kg BW in the United States and 7,500 IU/kg BW in Canada, although higher doses in the range of those used in the study are used in practice. Blood samples were collected at fixed time intervals from 0.25 h to 12 days following final injection and analyzed to determine pharmacokinetic parameters. The highest C_{max} , 4.24 ± 1.08 mg/L, was observed for IM injection of the single dose in the neck, which also provided the shortest $t_{½}$, 8.85 h. Maximum concentration appeared in all cases at 5-6 h following administration. Injection (IM) in the neck gave a higher AUC, 73.03 ± 8.57 μg·h/mL, than IM injection in the gluteal muscle (62.04 ± 3.34 μg·h/mL) or SC injection in the neck (63.15 ± 6.25 μg·h/mL) at the 66,000 IU/kg BW dose. The $t_{½}$ was 15.74 and 15.96 for the IM injections in the gluteal muscle and 18.08 for SC injection in the neck, indicating some variability in the effectiveness of different modes of administration and injection locations.

Another study was reported (Papich *et al*, 1994) in which yearling steers were divided into groups of 4 animals. Group A received an IM injection of a 1:1 mixture of benzathine benzylpenicillin and procaine benzylpenicillin at 9000 IU/kg BW, Group B received an IM injection of 24,000 IU/kg BW of the same mixture in the gluteal muscle, while Group C was administered this mixture as a SC injection of 8,800 IU/kg BW in the neck muscle. Group D received an IM injection of benzathine benzylpenicillin alone in the gluteal muscle at 12,000 IU/kg BW. Blood samples were collected at regular intervals starting at 0.25 h following injection and extending to 14 days. For the combined formulations, C_{max} was observed within 1 to 4 h after treatment, and $t_{½}$ was from 40.6 to 57.7 h for all IM injections. For the SC injection, $t_{½}$ was 29.3 ± h, while for benzathine benzylpenicillin administered alone by IM injection, T_{max} was at 17.6 ± 6.1 h. AUC varied with the dose and mode of injection, being 0.17 μg·d/mL for the SC injection and, for the IM injections, 0.35 μg·d/mL (9000 IU/kg), 0.97 μg·d/mL (24,000 IU/kg) and 0.65 μg·d/mL (benzathine benzylpenicillin only). The results demonstrated the faster uptake of procaine benzylpenicillin relative to benzathine benzylpenicillin and also the variability in pharmacokinetic parameters associated with IM and SC injection of the same product.

Nine calves (149-301 kg BW) were treated in a three-way, randomized crossover experiment with washout periods of at least one week with three different commercially available formulations containing procaine benzylpenicillin and dihydrostreptomycin (Groen *et al.*, 1996). Each formulation, which contained 200,000 IU/mL of procaine benzylpenicillin and 150-200 mg/mL dihydrostreptomycin, was administered at a dose of 0.1 mL/kg BW by IM injection. Pharmacokinetic parameters for benzylpenicillin as measured in serum from blood samples collected from 0.5 to 72 h following treatment were similar for the three formulations, with no statistical difference observed in AUC_{0-t} (mean 13.2- 13.4 μg.h/mL) or $AUC_{0-\infty}$ (mean 13.7-14.0 μg.h/mL). There were statistical differences in the parameters which reflect absorption rate, with $t_{½}$ varying from 5.5 ± 2.7 to 8.3 ± 2.9 h and C_{max} varying from 1.09 ± 0.41 to 1.53 ± 0.48 μg/mL. The formulation which was absorbed at the slowest rate caused the most tissue damage, based on measurements of creatine phosphokinase kinetics.

Horses

Five horses received procaine benzylpenicillin at 20,000 IU/kg BW in 5 different sites of injection: (1) SC in the pectoral area; (2) IM in the belly; (3) IM in musculus serratus ventralis cervicus; (4) IM in the biceps muscle, mid-way between the hip and knee; and (5) IM in the gluteus muscle (Firth *et al*, 1986). Highest $AUC_{0\text{-}12\ h}$ (26.0 ± 10.2 IU·h/mL) and shortest $t_{½}$ (8.0 ± 2.7 h) were observed for treatment (3). Subsequently, it was also demonstrated that administration of phenylbutazone boosted levels of benzylpenicillin in plasma when administered concurrently with an IM injection of procaine benzylpenicillin (Firth *et al*, 1990).

Procaine benzylpenicillin was administered IM at 12 mg/kg BW to six ponies and concentrations of benzylpenicillin in plasma and tissue chamber fluid were measured at intervals up to 24 h after treatment (Ensink *et al*, 1996). The AUC in μg·h/mL was 8.8 ± 2.0 for plasma and 4.8 ± 1.7 for tissue chamber fluid, with t_{max} of 3.5 ± 0.8 h and 12.3 ± 9.7 h, respectively. In this study, it was noted that, while renal elimination of benzylpenicillin is rapid, treatment IM with procaine benzylpenicillin can result in a maximum residence time of up to 10 h for benzylpenicillin in plasma. Concentrations in the

tissue chamber fluid exceeded those in the plasma only after C_{max} has been reached and concentrations in both compartments were declining.

Rabbits

Four *P. multocida* free and four infected rabbits each received a single IM injection of procaine benzylpenicillin at 60,000 IU/kg BW (Welch *et al*, 1987). Blood was collected from each animal at 0, 1, 3, 5, 8, 16 and 24 h following injection and nasal washings were collected at 0, 4, 9 and 24 h. Higher concentrations of benzylpenicillin were found in the blood samples collected from the infected rabbits than in the non-infected rabbits in the 1, 3, 5 and 8 h samples. In infected rabbits, the maximum concentration in serum was 5.86 ± 1.09 mg/L at 1 h, declining to 1.90 ± 0.88 mg/L at 8 h. In the non-infected rabbits a maximum concentration of 2.34 ± 1.51 mg/L was seen at 3 h, declining to 0.53 ± 0.24 mg/L at 8 h. A similar profile was observed in nasal washings, where concentrations declined from 0.06 mg/L at 4 h to 0.04 mg/L at both 9 and 24 h in the infected rabbits, but increased from 0.02 mg/L at 4 h to 0.06 mg/L at both 9 and 24 h in non-infected rabbits.

Twelve female rabbits were treated with three commercial products containing procaine benzylpenicillin (120 mg/mL) and dihydrostreptomycin (150-200 mg/mL) in a 4-way, randomized crossover experiment in which the rabbits were divided into 4 groups of 3 animals each (Groen *et al*, 1996). One group received, intravenously, a mixture prepared in the laboratory containing procaine benzylpenicillin and dihydrostreptomycin, while the remaining groups were each treated by IM injection, respectively, with one of the three commercial products. For the benzylpenicillin in the three commercial products, $AUC_{0-\infty}$ was 817 ± 145 to 867 ± 124 μg·min/mL, but $t_{½}$ varied from 111 ± 49 to 374 ± 279 min. C_{max} decreased from 4.4 ± 1.2 to 2.1 ± 0.9 μg/mL as elimination halflife increased, demonstrating that different formulations will have differences in elimination profile.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

No studies using radiolabeled procaine benzylpenicillin were found during the period covered by this literature survey (1984 - 1997).

Other Residue Depletion Studies (with Unlabelled Drug)

Cattle

Six groups (3 animals per group) of yearling steers received an IM injection of 24,000 IU/kg BW procaine benzylpenicillin on 5 successive days, after which the groups were slaughtered, respectively, at 1, 2, 3, 4, 8 and 12 days after the last injection (Korsrud *et al*, 1993). The experiment was repeated using a dose of 66,000 IU/kg BW, but adding two groups of 4 animals each, which were killed, respectively, at 10 and 16 days following the final injection of procaine benzylpenicillin. The results were compared with another experiment in which four groups of 3 yearling steers each received 66,000 IU/kg BW procaine benzylpenicillin by SC injection, again repeated over 5 successive days. Finally, six steers were injected SC with 66,000 IU/kg BW procaine benzylpenicillin, with each steer receiving several injections spaced at timed intervals so that, at the time of slaughter, injection sites were obtained which were 10, 15, 20 or 30 days old. Tissues were analyzed for benzylpenicillin residues using a high performance liquid chromatographic (HPLC) method of analysis with a detection limit of 0.005 mg/kg. Residues in edible tissues resulting from the administration of procaine benzylpenicillin IM at 24,000 and 66,000 IU/kg BW are reported in Table 1. Residues were below the detection limit in many of the injection sites collected at slaughter from the animals treated at 24,000 IU/kg BW and ranged from <0.005 mg/kg to 1.20 mg/kg in injections sites from the animals which received 66,000 IU/kg BW.

There was no clear correlation between time from treatment to slaughter and residues found at the injection site for IM administration. However, in the treatment groups which received procaine benzylpenicillin IM at 66,000 IU/kg BW, two cases of drug entrapment in the musculature at the injection site were encountered. One resulted in residues of 1.20 mg/kg 10 days following treatment, while the other injection site area contained 0.44 mg/kg of benzylpenicillin at 16 days following injection. These injection sites would not have been readily detected in a routine post-mortem inspection and were attributed to the use of injection volumes in excess of 30 mL of the formulated product.

Residues resulting from the SC administration of procaine benzylpenicillin, which were higher than those seen for IM administration, fell to below 0.05 mg/kg in both diaphragm and gluteal muscle 3 days after final treatment, but remained

above this concentration in kidney and liver at 4 days post-treatment. SC administration also resulted in visible deposits of the drug at the injection site at slaughter, with the injection area characterized by edema and haemorrhage. These sites were clearly visible at slaughter and there was a trend to lower residues at the injection site with elapsed time from treatment to slaughter. However, at 10 days following treatment, one injection site was found to have residues of 3.60 mg/kg. As in the case of IM administration, with the exception of injection sites, highest residues were found in the liver, followed by kidney and muscle.

Table 1. **Residues in tissues resulting from IM administration of procaine benzylpenicillin at 24,000 or 66,000 IU/kg BW, and from SC administration at 66,000 IU/kg BW, on 5 successive days in yearling steers.**

Withdrawal Period (d)	Body Weight (kg)	Dose [1]	Benzylpenicillin Residues in Tissues (mg/kg)			
			Kidney	Liver	Diaphragm Muscle	Gluteat Muscle[2]
1	444±8	A	1.10±0.62	2.00±0.28	0.04±0.00	0.03±0.02
	463±2	B	2.80±0.49	2.30±0.41	0.15±0.02	0.08±0.02
	469±23	C	1.60±0.20	4.70±0.43	0.29±0.02	0.10±0.01
2	494±8	A	0.65±0.39	0.35±0.24	0.03±0.02	0.06±0.03
	465±13	B	1.00±0.34	1.60±0.29	0.05±0.01	0.04±0.02
	466±6	C	0.83±0.18	2.50±0.71	0.07±0.02	0.05±0.00
3	582±10	A	0.03±0.02	0.02±0.02	<0.005	<0.005
	498±11	B	0.24±0.11	0.37±0.18	0.005±0.0050	0.009±0.009
	463±9	C	0.50±0.24	0.88±0.34	0.007±0.004	0.014±0.004
4	421±31	A	0.02±0.00	0.02±0.01	<0.005	<0.005
	451±37	B	0.01±0.01	0.05±0.02	<0.005	<0.005
	472±9	C	0.39±0.10	0.48±0.10	0.013±003	<0.005
8	472±36	A	<0.005	<0.005	<0.005	<0.005
	466±17	B	0.01±0.00	0.07±0.04	<0.005	<0.005
10	550±4	B[3]	0.01±0.01	0.03±0.01	NA[4]	<0.005
12	439±42	A	<0.005	<0.005	<0.005	<0.005
	404±16	B	<0.005	<0.005	<0.005	<0.005
16	607±7	B[3]	0.01±0.01	0.01±0.01	NA[4]	<0.005

[1] Dose: A, 24,000 IU/kg BW IM; B, 66,000 IU/kg BW IM; C, 66,000 IU/kg BW SC
[2] Collected from side of animal where drug was not injected.
[3] These groups contained 4 animals; all others contained 3.
[4] NA indicates "not analyzed".

Subsequently, a depletion study was reported in which cattle were administered either a combination of procaine benzylpenicillin and benzathine benzylpenicillin (1:1) IM at 8,600 IU/kg BW or SC at 8,800 IU/kg BW, or benzathine benzylpenicillin alone IM at 12,000 IU/kg BW (Korsrud *et al*, 1994). Treatment groups were as follows: 10 steers each received a single IM dose of 8600 IU/kg BW benzathine benzylpenicillin - procaine benzylpenicillin (1:1), the approved dose in Canada, and were slaughtered in groups of 5 at 14 and 30 days, respectively, following administration. Five other steers each received a single SC injection of 8800 IU/kg BW benzathine benzylpenicillin - procaine benzylpenicillin (1:1), an approved dose in the United States, and were slaughtered at 30 days following treatment. Fifteen steers were administered 24,000 IU/kg BW benzathine benzylpenicillin - procaine benzylpenicillin (1:1) IM, in two injections of equal volume, separated by 6-8 cm, but in the same general injection site at the same time, after which they were killed in groups of 5 at 8, 14 and 50 days following treatment. Finally, seven steers received 12,000 IU/kg BW benzathine benzylpenicillin,

as 3 injections of equal volume in a triangular pattern, 6-8 cm apart, administered at the same time, following which the steers were killed at day 14. Several untreated steers served as controls. Samples were analyzed using the same HPLC method as in the previous study. Using the recommended dose, benzylpenicillin residues were detectable in liver (0.007 ± 0.004 mg/kg) 14 days following treatment IM of steers with the mixed benzylpenicillins and at 30 days following SC administration (0.013 ± 0.005 mg/kg). Residues in injection sites following IM administration were 3.20 ± 0.72 mg/kg at 14 days and 2.10 ± 0.86 mg/kg at 30 days, while residues at SC injection sites were 1.60 ± 1.00 mg/kg at day 30. At the 24,000 IU/kg BW dose of the mixed benzylpenicillins, residues in IM injection sites were 104.00 ± 55.6 mg/kg at day 14 and 1.20 ± 0.38 mg/kg at day 50. Following SC administration of benzathine benzylpenicillin at 12,000 IU/kg BW, injection sites contained 7.8 ± 1.12 mg/kg benzylpenicillin at day 14. In all treatment groups, residues in liver were below 0.05 mg/kg at day 14. The results demonstrated that benzathine benzylpenicillin results in persistent residues at injection sites when used alone or in combination with procaine benzylpenicillin and that the residues observed were substantially higher than those seen when procaine benzylpenicillin was administered alone, even at higher doses.

Swine

Four groups of 6 market hogs (approx. 90 kg BW) were fed a diet containing a combination of sulfamethazine (330 mg/kg diet), chlortetracycline (330 mg/kg diet) and procaine benzylpenicillin (165 mg/kg diet), a dose 3 times that approved in Canada (Korsrud *et al.*, 1996). The groups were slaughtered, respectively, at withdrawal times of 0, 2, 4 and 8 days, with food access being denied for 5 h prior to slaughter. A fifth group of hogs received non-medicated feed and served as controls. Tissue samples were analyzed by an HPLC method with an LOD of 0.005 mg/kg for liver, kidney and muscle. Benzylpenicillin residues were detected in kidney from only one of the hogs in the zero withdrawal group (0.018 mg/kg), with all other samples from the other hogs in the study containing no detectable penicillin residues.

Groups of six market weight pigs (approx. 90 kg BW) each received IM 15,000 IU/kg BW procaine benzylpenicillin per pig on 3 successive days, following which the groups were slaughtered at 1, 2, 3, 4 and 8 days after final treatment (Korsrud *et al.*, 1998). Two other groups containing 6 and 7 pigs, respectively, received the same treatment and were slaughtered at 5 days after final administration. Samples were analyzed using the same HPLC method as in the previous study with pigs. Highest residues were found in kidney samples, followed by skin, muscle and fat. Liver was not tested for residues in this study. No significant residues were found in injection sites collected from animals slaughtered at 8 days following treatment. Residues found in tissue samples are summarized in Table 2.

Table 2. **Benzylpenicillin residues in tissues collected at slaughter from pigs which received procaine benzylpenicillin IM at 15,000 IU/kg BW on three successive days.**

Days Post-Treatment	Benzylpenicillin Residues in Tissues (mg/kg)					
	Kidney	Muscle	Skin	Fat	Injection Site (right neck)	Injection Site (left neck)
1	1.30±0.41	0.03±0.01	0.08±0.03	0.01±0.00	---[a]	---[a]
2	0.12±0.07	<0.005	0.02±0.01	<0.005	---[a]	---[a]
3	0.24±0.18	<0.005	<0.015	<0.005	30.0±14.6	---[a]
4	0.006±0.004	<0.005	0.02±0.01	<0.005	0.10±0.08	0.01±0.01
5	0.005±0.005	<0.005	<0.015	<0.005	1.10±1.05	<0.005
5 (repeat)	<0.005	<0.005	<0.015	---[b]	<0.005	0.01±0.01
8	<0.005	<0.005	<0.015	---[b]	<0.005	<0.005

[a] No sample collected; [b] Not analyzed.

Chickens

Four experimental treatments were randomly each assigned to two out of eight pens in which 400 day-old broiler chicks had been distributed at random, 50 chicks per pen (Proudfoot *et al*, 1993). The four treatments, which continued for 42 days until slaughter, were a control diet with no benzylpenicillin added, a diet containing 27.5 mg/kg of procaine benzylpenicillin, a diet with procaine benzylpenicillin provided via drinking water at approximately 27.5 mg/kg diet equivalent, and a replicate of the preceding with the procaine benzylpenicillin concentration reduced by one-half. The

concentrations of benzylpenicillin used in these experiments were significantly higher than the recommended dose of 2.2 mg/kg. Kidney, liver and muscle samples from the birds which were provided the diet which included 27.5 mg/kg procaine benzylpenicillin (Group 2) were tested for benzylpenicillin residues using a thin-layer chromatography-bioautography analytical method with a limit of detection (LOD) of 0.01 mg/kg for benzylpenicillin. No detectable residues were found in any of the tissues.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The 36th meeting of the Committee, in reviewing benzylpenicillin, concluded that there were good, sensitive bioassay methods for measuring residues in milk and in meat at the concentrations of interest. It was also noted that these methods were not specific for benzylpenicillin and required confirmation by liquid chromatographic methods or mass spectral methods, which had not been demonstrated to have the required sensitivity. This methodology review has therefore been confined to methods published since 1990.

Liquid chromatographic, mass spectral and other physicochemical methods for residue analysis do not distinguish between the various formulated products, which include procaine, benzathine and the sodium and potassium salts of the target analyte, benzylpenicillin. Several extensive reviews have been published since 1990 that provide an excellent summary of the available residue methods (Boison, 1992; Boison, 1995). Of the methods discussed in these references, two were reviewed and accorded provisional status by the Codex Committee on Residues of Veterinary Drugs in Foods. These are, respectively, a gas chromatographic (GC) method of analysis (Meetschen and Petz, 1990) and an HPLC assay (Boison *et al.*, 1991). The HPLC method is based on solid phase extraction and derivatization with 1,2,4-triazole-mercuric chloride, which forms a methylmercaptide derivative of benzylpenicillin which possesses a strong uv-absorbance at 325 nm. Both of these methods were considered to have demonstrated sufficient analytical sensitivity to meet the requirements for regulatory authorities to monitor compliance with the MRLs adopted by the 36th meeting of the Committee.

While an exhaustive review of published methods will not be undertaken in this report, several recent methods, not included in the Boison reviews, should be mentioned. These include an HPLC method for benzylpenicillin residues in milk, with an LOQ of 0.004 mg/L and an average recovery of 82% (Hormazábal and Yndestad, 1995), a method based on gel electrophoresis (Cutting *et al*, 1995) and a multi-residue HPLC method for β-lactams in milk (Moats and Harik-Khan, 1995). In addition, advances in mass spectral equipment and techniques have resulted in improved confirmatory methods which meet the sensitivity requirements for confirmation at the MRLs (Straub *et al*, 1994; Blanchflower *et al*, 1994). Various test kits are also now commercially available for the detection of β-lactams which have the required sensitivity for use in a regulatory program (Boison and MacNeil, 1995).

The major barrier to multi-laboratory validation of analytical methods is the limited stability of benzylpenicillin residues in samples of animal tissues, even when stored at –20°C (Boison *et al*, 1992). In this study, significant loss of residue was observed in samples after 10 days of frozen storage in liver, kidney and muscle tissues frozen without pre-homogenization. Subsequently, it was demonstrated that an accelerated rate of loss of residue occurs in liver samples which have been homogenized prior to storage, with more rapid loss observed in spiked samples than for incurred tissue residues (Gee *et al*, 1996). This latter finding is of particular significance as normal practice for an interlaboratory trial is to prepare sets of samples from homogenized pools of tissue.

APPRAISAL

Procaine benzylpenicillin is one of a number of available formulations of benzylpenicillin, which was previously reviewed by the 12th and 36th meetings of the Committee. A maximum daily intake of 30 μg of residues of benzylpenicillin, based on hypersensitivity reactions of allergic individuals, and MRL's of 0.05 mg/kg for liver, kidney and muscle (all species) and 0.004 mg/kg for milk have been recommended. The 36th meeting of the Committee noted the limited availability of chemical assay methods and made the following recommendations:

1. The provision of further information and the results of new studies on the depletion of residues of benzylpenicillin from the edible tissues of food-producing animals.
2. Investigation of the accuracy and precision of the assays used to measure residues of penicillin.
3. The development of more sensitive chemical assays for benzylpenicillin.

Pharmacokinetic data

In the studies which follow 1 mg of procaine benzylpenicillin equates with 1,667 IU of the drug.

No data were available for review on studies with laboratory animals or on studies using radiolabeled procaine benzylpenicillin, but a number of non-GLP studies involving food animals, some conducted by regulatory authorities, had been published since benzylpenicillin was last reviewed by the Committee.

Cattle In five calves (80-110 kg BW), which received procaine benzylpenicillin as a single IM injection at a dose of approximately 18000 IU/kg BW, the plasma C_{max} was 2.1 IU/mL with an elimination half-life $t_{½}$ of 4.3 h and AUC of 18.25 IU/mL/h. Decline in concentrations of benzylpenicillin in plasma was uniphasic. When six calves (102 -120 kg BW) with implanted tissue cages received procaine benzylpenicillin at 30 mg/kg BW (approx. 30,000 IU/kg BW) by IM injection in the neck, $t_{½}$ was 2.98 h in serum and 10.21 h in tissue cage fluid. C_{max} was 5.37 µg/mL in serum at 1.5 h after injection, and 1.52 µg/mL in tissue cage fluid at 7.7 h.

In six cattle which received procaine benzylpenicillin IM at 10,000 IU/kg BW, in combination with dihydrostreptomycin (12.5 mg/kg BW), the elimination half-life in serum was 1.87 h. When procaine benzylpenicillin was administered IM to six mature cows at 20,000 IU/kg BW, then 7 days later using the same dose by SC injection, repeated on 3 successive days, C_{max} in serum was 30 min after IM administration and 2 h after SC injection. Concentrations of benzylpenicillin in plasma decreased more rapidly in the first 24 h following SC injection, when compared to IM injection. Benzylpenicillin residues in tissues of the animals that were sacrificed 5 days after the final SC injection were distributed as follows: liver, 1.00 mg/kg; kidney (renal cortex), 0.90 mg/kg; kidney (renal medula), 0.58 mg/kg; muscle (diaphragm), 0.13 mg/kg; muscle (gluteal), 0.10 mg/kg; fat, 0.06 mg/kg; muscle adjacent to injection site, 1.15 mg/kg.

In steers which received procaine benzylpenicillin IM or SC at doses up to 66,000 IU/kg BW, in some cases on 5 successive days, the highest C_{max} , 4.24 µg/mL was observed for IM injection of a single dose in the neck. This treatment also provided the shortest plasma elimination half-life, $t_{½}$, of 8.85 h. C_{max} in all treatments occurred within 5-6 h following injection.

The pharmacokinetics of procaine benzylpenicillin used in combination with benzathine penicillin (1:1), administered IM or SC to yearling steers, was compared with IM injection of benzathine benzylpenicillin alone. C_{max} was observed within 1 to 4 h after treatment for the combined formulations, and $t_{½}$ was from 40.6 to 57.7 h for all IM injections. For the SC injection of the combined formulation, $t_{½}$ was 29.3 h. AUC varied with the dose and mode of injection, from 0.17 µg·d/mL for the SC injection to 0.35 - 0.97 µg·d/mL for IM injection of the combined formulation and 0.65 µg·d/mL for benzathine benzylpenicillin alone. The results demonstrated the variability in pharmacokinetic parameters associated with the use of different formulations, and with IM or SC injection of the same product.

When nine calves (149-301 kg BW) were treated in a three-way, randomized crossover experiment with three different commercially available formulations containing procaine benzylpenicillin (200,000 IU/mL) and dihydrostreptomycin (150-200 mg/mL) administered at a dose of 0.1 mL/kg BW by IM injection, no statistical difference was observed in AUC_{0-t} (mean 13.2-13.4 µg·h/mL) or $AUC_{0-\infty}$ (mean 13.7-14.0 µg.h/mL) in serum samples collected from 0.5 to 72 h after treatment. There were significant differences in $t_{½}$, which varied from 5.5 to 8.3 h, and in C_{max}, which ranged from 1.09 to 1.53 µg/mL.

Horses In horses which received procaine benzylpenicillin at 20,000 IU/kg BW IM or SC in different muscle groups, highest AUC_{0-12h} (26.0 IU·h/mL) and shortest $t_{½}$ (8.0 h) were observed for injection in the front shoulder. Six ponies were administered procaine benzylpenicillin IM at 12 mg/kg (equivalent to 12,000 IU/kg) BW, resulting in an AUC, in µg·h/mL, of 8.8 for plasma and 4.8 for tissue cage fluid, with t_{max} of 3.5 h and 12.3 h, respectively. Renal elimination of benzylpenicillin was rapid, with a maximum residence time of 10 h for penicillin in plasma, while concentrations in the tissue cage fluid exceeded those in the plasma only after C_{max} had been reached and concentrations in both compartments were decreasing.

Rabbits The difference in pharmacokinetics of procaine benzylpenicillin in healthy and sick animals was demonstrated when *P. multocida* free and infected rabbits received a single IM injection of procaine benzylpenicillin at 60,000 IU/kg BW. Higher concentrations of benzylpenicillin were found in blood samples collected from the infected rabbits than from the non-infected rabbits up to 8 h following treatment. In infected rabbits, serum C_{max} was 5.86 µg/mL at 1 h, declining to 1.90 µg/mL at 8 h, while in the non-infected rabbits C_{max} was 2.34 µg/mL at 3 h, declining to 0.53 µg/mL at 8 h. When rabbits

were treated with thee commercial products containing procaine benzylpenicillin and dihydrostreptomycin in a 4-way, randomized crossover experiment similar to the study with calves, $t_{½}$ varied from 111 to 374 min and C_{max} decreased from 4.4 to 2.1 g/mL as elimination halflife increased, demonstrating again that different formulations provide differences in elimination profile.

No information was available on the pharmacokinetics following oral administration of procaine benzylpenicillin.

Residue data

Cattle A study was conducted in which yearling steers received an IM injection of 24,000 or 66,000 IU/kg BW procaine benzylpenicillin on 5 successive days, or 66,000 IU/kg BW procaine benzylpenicillin by SC injection, again repeated over 5 successive days. In addition, another group of steers received procaine benzylpenicillin SC at 66,000 IU/kg BW, with each steer receiving several injections spaced at timed intervals so that injection sites were collected which were 10, 15, 20 or 30 days old at slaughter. Tissues were analyzed for benzylpenicillin residues using a liquid chromatographic method of analysis with a detection limit of 0.005 mg/kg. Mean residues were <0.05 mg/kg in all tissues for the 24,000 IU/kg BW treatment group at day 4, while 10 days were required to reach this concentration range for the 66,000 IU/kg BW IM treated animals. Residues were more persistent following SC injection at 66,000 IU/kg BW, with observed distribution as follows at 4 days withdrawal: liver, 0.48 mg/kg; kidney, 0.39 mg/kg; diaphragm muscle, 0.013 mg/kg; gluteal muscle, <0.005 mg/kg. Residues were <0.005 mg/kg in many of the injection sites collected at slaughter from the animals treated at 24,000 IU/kg BW and ranged from <0.005 mg/kg to 1.20 mg/kg in injection sites from the animals which received 66,000 IU/kg BW. While there was no clear relationship between time from treatment to slaughter and residues found at the injection site following IM administration, several instances of drug entrapment in the musculature at the injection site were noted, one resulting in residues of 1.2 mg/kg at 10 days following treatment, while another injection site contained 0.44 mg/kg of benzylpenicillin at 16 days following the final injection. These injection sites would not have been readily detected in a routine post-mortem inspection and were attributed to the use of injection volumes in excess of 30 mL of the formulated product. Administration of the drug by SC resulted in visible deposits of the drug at the injection site at slaughter, with the injection area characterized by edema and haemorrhage. Excluding injection sites, highest residues were found in the liver, followed by kidney and muscle, for both IM and SC injection.

In a subsequent study, cattle were administered either a combination of procaine benzylpenicillin and benzathine benzylpenicillin (1:1) IM or SC, or benzathine benzylpenicillin alone IM. Using the label dose (8,600 IU/kg BW IM or 8,800 IU/kg BW SC), residues were detectable in liver (0.007 mg/kg) 14 days following IM treatment of steers with the mixed penicillins and at 30 days following SC administration (0.013 mg/kg). Residues in injection sites following IM administration were 3.20 mg/kg at 14 days and 2.10 mg/kg at 30 days, while residues at SC injection sites were 1.60 mg/kg at day 30. At a dose of 24,000 IU/kg BW IM of the mixed penicillins, residues in injection sites were 104 mg/kg at day 14 and 1.2 mg/kg at day 50. Following SC administration of benzathine penicillin alone at 12,000 IU/kg BW, injection sites contained 7.8 mg/kg penicillin at day 14. In all treatment groups, residues in liver were below 0.05 mg/kg at day 14. The findings demonstrated that the risk of persistent residues at injection sites increases when high doses of long-acting formulations are administered.

Pigs In pigs (approx. 90 kg BW) fed a diet containing a combination of sulfamethazine (330 mg/kg diet), chlortetracycline (330 mg/kg diet) and procaine benzylpenicillin (165 mg/kg diet), benzylpenicillin residues were detected in kidney from only one of the hogs in the zero withdrawal group (0.02 mg/kg), with all other tissue samples containing no detectable benzylpenicillin residues. Analysis was by a liquid chromatographic method with an LOD of 0.005 mg/kg for liver, kidney and muscle.

In another study, pigs (approximately 90 kg BW) each received IM 15,000 IU/kg BW procaine benzylpenicillin per pig on 3 successive days. Groups were sacrificed at 1, 2, 3, 4, 5 and 8 days after final treatment. Residues were determined using a liquid chromatographic method with a LOD of 0.005 mg/kg in tissues. Highest residues were found in kidney samples, ranging from 1.30 mg/kg at day 1 to 0.24 mg/kg at day 3 and <0.005 mg/kg at day 8. The only other tissue samples in which residues exceeded 0.05 mg/kg was skin at day 1 (0.08 mg/kg). Liver was not tested for residues in this study and no residues above the MRL were found in injection sites collected from animals slaughtered at 4-8 days following treatment.

Chickens Day-old broiler chicks received a diet for 42 days containing 27.5 mg/kg of procaine benzylpenicillin, or an equivalent dose via drinking water, with no detectable residues found in any of the tissues (kidney, liver, muscle) tested using a thin-layer chromatography-bioautography analytical method with a limit of detection (LOD) of 0.01 mg/kg. The concentrations of benzylpenicillin used in these experiments were significantly higher than the recommended rate of 2.2 mg/kg.

Analytical methods

The 36th meeting of the Committee noted the availability of bioassay methods for measuring benzylpenicillin residues in milk with detection limits between 0.001 and 0.010 mg/L. Such methods were also available for residues in tissues at the concentrations of interest, but the Committee also observed that these methods were not specific for benzylpenicillin and required confirmation by liquid chromatographic methods or mass spectral methods. The available chemical methods had detection limits of 0.05-0.10 mg/kg for tissues and 0.01-0.05 mg/kg for milk, so lacked the required sensitivity.

The present Committee noted that for liquid chromatography, mass spectrometry and other physicochemical methods for residue analysis, the target analyte is typically benzylpenicillin. These methods do not usually distinguish between the various formulated products, which include procaine, benzathine and the sodium and potassium benzylpenicillin salts. It was further noted that several comprehensive reviews have been published since 1990 which provide an excellent picture of the available residue methods for benzylpenicillin, and that two methods have met the criteria for provisional methods established by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)(Vol. 3, Codex Alimentarius). Both of these methods were considered to have demonstrated sufficient analytical sensitivity to meet requirements for regulatory authorities accepting the MRL's adopted by the 36th meeting of the Expert Committee. The Committee considered that a number of methods may now be found in the scientific literature, using various analytical techniques, including liquid chromatography and gel electrophoresis, and that some of these methods have limits of detection of 0.002 mg/L for milk and 0.005 mg/kg or less for edible tissues. Some such methods may be suitable for further consideration by the CCRVDF. It also noted that there have been significant improvements in the sensitivity of mass spectrometry techniques, making confirmation of residues at the recommended MRLs of 0.004 mg/L in milk and 0.05 mg/kg in edible tissues feasible with current equipment. A variety of rapid test techniques using bioassay or ELISA are also available for screening purposes.

The Committee, however, noted that a major barrier to multi-laboratory validation of analytical methods is the limited stability of benzylpenicillin residues in samples of animal tissues, even when these tissues are stored at -20 C. For compounds where stability is an impediment to multi-laboratory validation of a method using an exchange of samples, alternative approaches using data individually generated in multiple laboratories, or validation using other criteria acceptable to the CCRVDF, should be considered.

National regulatory authorities should note that different formulations and modes of administration, as well as the use of extra-label doses, may result in more persistent residues in excess of the MRLs, particularly in organ tissues and at injection sites when slow release formulations are used. Suitable analytical methods are available for application as screening, determinative or confirmatory tests in a regulatory monitoring program.

Maximum Residue Limits

The Committee considered that MRLs established by the 36th meeting of the Committee for benzylpenicillin remain appropriate and are applicable to residues resulting from the use of procaine benzylpenicillin. The MRL for liver, kidney and muscle in cattle, pigs and chickens is 50 µg/kg and 4 µg/L for milk. Based on available data, the recommended tissues for regulatory monitoring are kidney or liver, while muscle is an appropriate target tissue for testing for international trade purposes. Procaine benzylpenicillin is also used in horses, sheep, turkeys, rabbits, quail, and pheasants. Due to the lack of information, MRLs could not be established for those species.

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SARAFLOXACIN

First draft prepared by
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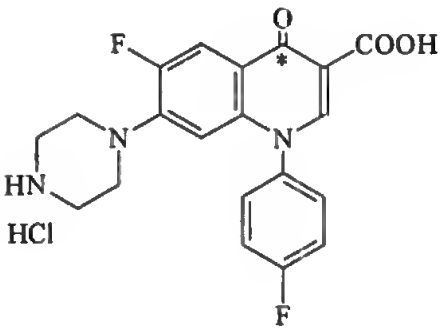
IDENTITY

Chemical name: Sarafloxacin hydrochloride; 6-fluoro-1-(4-fluorophenyl)-7-piperazinyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid hydrochloride.

Synonyms: Floxasol

Structure:



* ^{14}C-4 for radiometric studies >98% pure

Molecular formula: $C_{20}H_{18}ClF_2N_3O_3$

Molecular weight: 421.8 (hydrochloride) 385.4 (free base)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: sarafloxacin hydrochloride

Appearance: white to pale yellow powder

Melting point: >300°C

Solubility (g/L): water, 0.31; methanol, 3.2; ethanol, 0.23; 1*N* NaOH, 165; DMSO, 8.8, practically insoluble in 1M HCl, chloroform, hexane, toluene.

Ultraviolet maxima: 261 nm and 317 nm

Factors affecting stability: the hydrochloride is stable in neutral, acidic and basic solutions; sarafloxacin is stable for 30 days at 110°C; in solution, sarafloxacin can be degraded by light and oxidising agents.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Administered in drinking water for turkeys and broilers as an antibiotic compound.

Dosage

For chickens: 20 mg/L in water; equivalent to 4 mg/kg BW for chickens from 3 weeks of age.

For turkeys: 30 mg/L in water; equivalent to 4 mg/kg BW for turkeys from 7 weeks of age.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics

Toxicological Test Species

Compliance with Good Laboratory Practice principles was not required for these pharmacokinetic studies. The quality and design of this study was consistent with current scientific standards.

Mice

Three groups of twelve female mice per group were dosed with sarafloxacin ^{14}C-base as follows: Animals in the first two groups were given a single oral dose of 10 mg sarafloxacin/kg BW. One group received the drug by IV administration and the other via gavage. Animals in the third group were given a dose of 100 mg sarafloxacin/kg BW by gavage. Urine and feces were collected from the mice daily for three days.

Estimates of absorption of the parent drug were derived from data on 0 - 24 h urinary excretion. For the 10 mg/kg BW dose, 48% (range 27-73%) of the parent drug was absorbed. For the 100 mg/kg BW dose, 34% (range 29-38%) of the parent drug was absorbed.

Within 3 days after administration of a single IV dose of 10 mg/kg BW of sarafloxacin to mice, 49% of the ^{14}C-dose was excreted in the urine and approximately 44% was eliminated in the feces. Following oral administration of the same dose, urinary and fecal excretion accounted for approximately 25% and 80%, respectively. Mice given the 100 mg/kg BW oral dose eliminated 18% of that dose in the urine and 74% in the feces. Almost all of the radioactivity was excreted during the first 24 hours after either oral or IV administration (Volume 4a).

Rats

Six groups of Sprague Dawley rats (18/sex/group) were dosed with sarafloxacin as follows: One group of animals received a single IV dose of 20 mg/kg BW of sarafloxacin. Four groups of animals received a single oral dose of 20, 75, 275 or 1000 mg/kg BW of sarafloxacin. Animals in the sixth group received an oral dose of 1000 mg/kg BW of sarafloxacin daily for 14 consecutive days. Blood samples (4 rats/group) were collected just prior to dosing and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post dosing on day 1 for the groups receiving the single dose and on days 1 and 14 for the 14 day dosing group. Plasma and urine samples were assayed for sarafloxacin base by a HPLC method. The pharmacokinetic parameters determined from this study are presented in Table 1. A comparison of the 0 to infinity AUCs following a single IV or oral 20 mg/kg BW dose of sarafloxacin indicated that the bioavailability was approximately 12% at this dose level. A plot of the AUC vs dose is linear up to 275 mg/kg BW (Volume 4b).

Table 1. Pharmacokinetic parameters of sarafloxacin in rats.

Dose (route) (mg/kg BW)	V_d (l/kg)	$T_{½}$ (elim) (h)	T_{max} (h)	C_{max} (mg/L)	k_a (h^{-1})	k_e (h^{-1})	ABC (mL/min/kg)
20 (IV)	5.3	2.0	-	-	-	0.3	30
20 (oral)	60	3.0	1.0	0.3	3.0	0.3	270
75 (oral)	70	2.0	2.0	0.6	1.0	0.4	470
275 (oral)	250	7.0	2.0	0.9	2.0	0.1	420
1000 (oral)	400	6.0	1.0	2.0	2.0	0.1	820
1000 (oral)*	110	6.0	2.0	8.0	1.0	0.1	200

ABC is apparent body clearance. * once daily for 14 days.

Rabbits

The absorption, metabolism and excretion of ^{14}C-labelled sarafloxacin was studied in 3 month old female New Zealand white rabbits. Two groups of 3 animals per group were dosed orally, by gavage, with 10 mg/kg BW of ^{14}C-sarafloxacin base. A third group of 3 animals received this same dose by IV administration. Blood samples were collected at 1, 3, 6, 12 and 24 hours after oral administration from animals in one of the groups dosed orally. Urine and feces were collected daily for five days from animals in the other oral dose group and the IV dosed group. Within 5 days after oral administration about 11% of the dose was eliminated in the urine and approximately 79% was eliminated in the feces. Urinary excretion following IV administration was used to determine that approximately 16% of the oral dose was systemically absorbed (volume 4c).

Dogs

Three groups of 14 dogs/group (species, age, sex not stated) were administered daily oral doses of 5, 25 or 125 mg/kg BW sarafloxacin base by capsule. After one month 6 dogs/group were killed and plasma and cerebrospinal fluid were collected for HPLC analysis. The remaining dogs continued to be treated daily for a total of 90 days. The pharmacokinetic parameters determined from this study are presented in Table 2.

Table 2. Pharmacokinetics of sarafloxacin base after oral administration to dogs.

Dose (mg/kg)	Mean half-life (h)[1]			AUC (mg·h/L)		
	2 doses	24 doses	79 doses	2 doses	24 doses	79 doses
5	5	6	6	9	9	10
25	5	5	6	30	31	30
125	5	6	6	104	108	106

[1] Samples were taken 1, 3, 6, and 24 hours after dosing.

For the low and mid-dose groups, peak serum levels of sarafloxacin were found most often in samples taken 3 hours after dosing. In the high-dose group maximum serum levels were found in the majority of animals 6 hours after dosing. Therefore the true half lives may be overestimated and the AUC values may be underestimated for some of the high-dose animals. Dose normalization of the AUC provides a gauge of dose-proportionality of systemic exposure. The trend of decreasing values of approximately 2, 1 and 1 µg·h/mL per mg/kg for the 5, 25 and 155 mg/kg BW dose groups, respectively, suggests that absorption efficiency is reduced with increasing dose size.

In summary, these data suggest that the dispositional kinetics of sarafloxacin in the dog are independent of dosage size and treatment duration while absorption of sarafloxacin becomes less efficient with increasing dose size (Volume 4d).

Tissue distribution of ^{14}C-sarafloxacin base following a single oral 10 mg/kg BW dose was studied in four adult male beagle dogs. Levels of radioactivity in tissues measured at 2 and 6 h after dosing are shown in Table 3 (Volume 4e).

Table 3. Levels (mg equivalents/kg or L) of radioactivity in tissues of male dogs after oral administration of ^{14}C-sarafloxacin base (Dose = 10 mg/kg BW)

Tissue	2 h	6 h	24 h	Tissue	2 h	6 h	24 h
Liver	14	12	2	Bone[2]	3	3	2
Kidney	16	14	1	Retina/uvea	15	43	45
Lung	6	5	1	Blood	3	3	0.4
Brain	0.4	0.7	0.3	Bile	154	454	420
Fat[1]	0.6	0.5	0.6	Urine	89	412	188
Muscle[1]	5	6	1				

[1] Percent dose in muscle and fat calculated assuming those tissues represent 46% and 10% of BW, respectively.
[2] Rib including marrow

The bioavailability of an oral dose of 200 mg sarafloxacin base, equal to 19.6 mg/kg BW, was studied in six adult female dogs. Three different dosage forms were administered – suspension, solution or capsule. The bioavailability of the suspension and capsule were similar. Zero to 32 hour mean AUC values for these formulations were 27 and 23 μg·h/mL, respectively. The mean AUC for the solution was 52 μg·h/mL. The author reported results from other bioavailability studies that showed that, compared to an equal IV dose, the bioavailability of an oral 10 mg/kg BW dose of the solution ranged from 58 to 70%. The relationship between dose and bioavailability appears not to be linear or log linear for the capsule formulation. The suspension produced AUC values that were approximately one half of those obtained from solution, however the author states that, in another study, a lower dose of 10 mg/kg BW, the formulations were equivalent. The basis for the formulation differences was not readily apparent. The author also referenced data from a human clinical study in which capsules of the same lot given to dogs were administered. In humans, urine recoveries of sarafloxacin are considered an approximate estimate of absorption since urinary excretion is the predominant route of elimination in humans. The author referenced results from a human clinical study where urine recoveries ranged from 24% at 1.3 mg/kg bw to 10% at 10.4 mg/kg bw, indicating that absorption rates in humans are considerably lower than absorption rates seen in dogs (Volume 4f).

Humans

A single oral dose of 100, 200, 400 or 800 mg sarafloxacin was administered to 22 healthy male volunteers ranging in age from 20-39 years. Blood samples were taken at pre-test and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 28, 32 and 48 hours after dosing. Urine was collected at hours 0-4, 4-8, 8-12, 12-16, 16-24, 24-32 and 32-48. Compliance with Good Laboratory Practice principles was not required for this study. The quality and design of this study was consistent with current scientific standards.

Plasma drug levels peaked 1.5-4 hours after dosing and declined biphasically, with the terminal phase becoming dominant approximately 12 h postdosing. The means of the individual peak levels for the 100, 200, 400 and 800 mg dose groups were 140, 180, 240 and 350 ng/ml. respectively. The corresponding dose-normalized peak levels were 106, 62, 44 and 34 ng/mL per mg/kg. Dose normalized AUC values for the 100, 200, 400 and 800 mg groups averaged 860, 570, 410 and 350 ng·h/mL per mg/kg, respectively. The declines in the dose normalized AUC and peak level values as a function of dose provide evidence that the efficiency of absorption deceased by a factor of about 3 as the dose was increased. Terminal phase half-lives averaged 9, 9, 10 and 11 hours for the 100, 200, 400 and 800 mg dose groups, respectively. The major route of elimination was renal excretion of unchanged drug. Renal clearances for the 100, 200, 400 and 800 mg groups averaged 280, 290, 290 and 260 mL/min, respectively. Intergroup variations were not significantly different. Urinary recoveries of unchanged drug averaged 19, 14, 10 and 7% of the administered 100, 200, 400 and 800 mg doses, respectively. The extent of absorption of sarafloxacin decreased from approximately 27 to 34% for the 100 mg dose to 11-13% for the 800 mg dose (Volume 47).

Food Animals

Chickens and Turkeys

Chickens and turkeys were administered ^{14}C-Sarafloxacin hydrochloride by gavage 4 times daily for 5 days. More than 79 - 89% of the dose was excreted within 6 h of dosing (Volume 57, Volume 59).

Metabolism

Mice and Rabbits

The biotransformation of sarafloxacin was investigated in the excreta of mice and rabbits following oral and IV administration in the studies described above (Volume 4a & 4c). In both species parent drug was the main component accounting for more than 80% of the administered dose. Sarafloxacin glucuronide was found as a minor residue (1 - 10% of dose) and N-acetyl-sarafloxacin, 3'-oxo-sarafloxacin- and two unknown compounds were isolated but were less than 1% of the dose.

Humans

The pharmacokinetics and metabolism of sarafloxacin in humans was studied. In this study, 2 groups of 6 volunteers were administered a single oral dose of 100 or 200 mg sarafloxacin and 2 groups of 5 volunteers were administered a single oral dose of 400 or 800 mg sarafloxacin.

The metabolism of sarafloxacin appears to mainly involve oxidative degradation of the piperazinyl substituent, first producing 3'-oxo-sarafloxacin (M3). Subsequent oxidation produces an ethylene diamine substituted quinolone (M5), which in turn is oxidized to an aminoquinolone (M4). The plasma level profiles of M5 parallel those of parent drug, however the AUC of M5 consistently averaged only about 6% of the AUC of sarafloxacin. The concentration of M4 in plasma and urine was considerably lower than that of M5. Due to its weak fluorescence, M3 was not detected in plasma.

In urine, the major drug related peak was sarafloxacin accounting for 75% to 80% of total urinary metabolites. Next to sarafloxacin the most predominant metabolite in urine was tentatively identified as M3. Its levels were typically 1/3 to 1/4 of the corresponding levels of sarafloxacin. Total urinary recoveries of parent drug plus metabolites were low and dose-dependent, changing from 2% to 10% as the dose increased from 100 to 800 mg. The extent of the decrease was similar to the decrease in the dose-normalized AUC. Collectively, M4, M5 and their conjugates accounted for less than 7% of the urinary pattern (Volume 47).

Chickens

Three male and three female chickens were administered 3.34 ± 0.26 mg/kg BW/day ^{14}C-Sarafloxacin HCl (34.4 µCi/mg) by gavage for 5 days. Livers were collected at 6 h after dosing and pooled for each sex. The liver samples were extracted with acidic and basic acetonitrile; 87% (female) and 85% (male) of the residues were extractable. The metabolic profiles were similar for male and female chicken extracts. The metabolites identified are shown in Table 4. This study was performed to GLP (Volume 57).

Turkeys

Three male and three female turkeys were administered 6.9 mg/kg BW/day by gavage ^{14}C-Sarafloxacin HCl (33.3 µCi/mg) for 5 days. Livers were collected at 6 h after dosing and pooled for each sex. The liver samples were extracted with acidic and basic acetonitrile; 83% of the residues were extractable. The metabolic profiles were similar for male and female turkey extracts. The metabolites identified are shown in Table 4.

Table 4. Metabolites of sarafloxacin in poultry liver

Component	% Total Residues in Turkeys		% Total Residues in Chickens	
	Male	Female	Male	Female
Sarafloxacin	20	21	69	65
Sarafloxacin sulphamic acid	7	6	8	13
Sarafloxacin glucuronide	20	25	8	13
Sarafloxacin sulphamic glucuronide	30	16	8	13
Others (4)	6	15	8	9

The main route of metabolism in poultry liver is the formation of either or both a sulphamic acid conjugate at the N-position in the piperazine ring or a glucuronide with the -COOH group. The unidentified minor metabolites were also conjugates because acid or base hydrolysis of the metabolites yielded parent Sarafloxacin. More conjugates were present in the turkey liver than chicken liver.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

All the studies examined the residues in equal numbers of both male and female birds. The analysis of the results indicated that there were no significant differences in the values for males or females; therefore the results for both sexes were combined.

Chickens

Chickens were administered 0.54 mg/kg BW ^{14}C-sarafloxacin hydrochloride by gavage four times daily for 5 days (Volume 57). The total dose per day was 2.2 mg per bird (3.4 mg/kg BW/day) which simulated 85% of the dose proposed for field use of the drug in drinking water (20 ppm). Groups of six birds were sacrificed at 6, 18, 36 and 72 h

after drug withdrawal. Light muscle, dark muscle, liver, skin with adhering fat, fat and kidney samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting (Volume 58). The results are shown in Table 5.
The residues were highest and most persistent in the liver tissue (Note; kidney not investigated). After one day of drug withdrawal the residues were only measurable in liver and skin. Three days after withdrawal no residues were detected in any of the tissues.

Table 5. Total residues, expressed as µg/kg equivalents of ^{14}C-sarafloxacin-hydrochloride in broilers after oral dosing with 3.4 mg/kg BW ^{14}C-sarafloxacin-hydrochloride per day for 5 days.

Tissue	6 h		18 h		36 h		72 h
	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	
Liver	221 - 482	322 ± 92	21 - 219	70 ± 75	17 - 28	21 ± 4	<LOD
Skin + Fat	19 - 39	29 ± 7	<LOD(4)-48	26 ± 11*	<LOD		<LOD
Fat	8 - 65	22 ± 21	<LOD		<LOD		<LOD
Light Muscle	24 - 45	35 ± 8	<LOD		<LOD		<LOD
Dark Muscle	18 - 38	28 ± 8	<LOD		<LOD		<LOD
Kidney	NM		NM		NM		NM

* LOD = 21 µg/kg used for determination of SD; NM = not measured.
LOD for light muscle = 5 µg/kg at 6 h, 22 µg/kg at 18, 36 and 72 h. LOD for dark muscle = 6 µg/kg at 6 h, 22 µg/kg at 18, 36 and 72 h. LOD for liver = 4 µg/kg at 6 h, 15 µg/kg at 18, 36 and 72 h. LOD for fat = 6 µg/kg at 6 h, 22 µg/kg at 18, 36 and 72 h. LOD for skin + fat = 5 µg/kg at 6 h, 21 µg/kg at 18, 36 and 72 h.

Turkeys

Turkeys weighing about 2.7 - 3.7 kg were administered 4.25 mg by gavage ^{14}C-sarafloxacin HCl four times daily for 5 days (Volume 59). The total dose per day was 21 mg per bird (ca. 7 mg/kg BW/day), which is higher than the recommended field dose of 4 mg/kg/day (30 ppm in drinking water) for turkeys of a similar age. Groups of six birds were sacrificed at 6, 18, 36 and 72 h after drug withdrawal. Light muscle, dark muscle, liver, skin with adhering fat, fat and kidney samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting. The results are shown in table 6.

Table 6. Total residues expressed as µg/kg equivalents of ^{14}C-sarafloxacin-hydrochloride in turkeys after oral dosing with 7 mg/kg/day ^{14}C-sarafloxacin-hydrochloride for 5 days.

Tissue	6 h		18 h		36 h		72 h	
	Range	Mean#	Range	Mean#	Range	Mean#	Range	Mean#
Liver	181 - 663	388 ± 175	65 - 108	87 ± 20	48 - 80	60 ± 11	25 - 43	35 ± 6
Fat	17 - 165	52 ± 56	<LOD(4)-33	27 ± 3*	<LOD		<LOD	
Skin + Fat	22 - 35	28 ± 5	<LOD(1)-28	22 ± 4*	<LOD(3)-26	19 ± 4*	<LOD(1) - 28	20 ± 5
Light Muscle	6 - 18	12 ± 3	<LOD		<LOD		<LOD	
Dark Muscle	6 - 14	12 ± 4	<LOD		<LOD		<LOD	
Kidney	NM		NM		NM		NM	

± standard deviation (SD) * LOD used for determination of SD. NM = not measured.
LOD for light muscle = 3 µg/kg at 6 h, 13 µg/kg at 18, 36 and 72 h. LOD for dark muscle = 3 µg/kg at 6 h, 12 µg/kg at 18, 36 and 72 h. LOD for liver = 3 µg/kg at 6 h, 15 µg/kg at 18, 36 and 72 h. LOD for fat = 6 µg/kg at 6 h, 25 µg/kg at 18, 36 and 72 h. LOD for skin + fat = 4 µg/kg at 6h, 16 µg/kg at 18, 36 and 72 h.

The residues were highest and most persistent in the liver tissue (Note; kidney not investigated). After 36 hours of drug withdrawal the residues were only measurable in liver and skin. Three days after withdrawal residues were still detected in all the liver samples and in 5 out of 6 skin tissues.

Residue Depletion Studies with Unlabelled Drug

Chickens

Broiler chickens weighing 1.84 -2.54 kg were given sarafloxacin in their drinking water for 119 hours at a concentration of 15.5 - 18.0 ppm (equiv. to 2.7 mg/kg BW / day). Groups of six birds were sacrificed at 0, 26, 96 and 122 h after drug withdrawal (Volume 51). Muscle, liver, lung, skin, fat and kidney samples were collected and the concentrations of sarafloxacin measured by HPLC (Volume 60b). The results are shown in Table 7. The mean values for males were higher than those for females for muscle, liver and kidney at time 0 h but there is no significant difference between the means. Thus the results for both sexes are combined.

Table 7. Residues (μg/kg) in broilers after administration of sarafloxacin at 15.5 – 18.0 mg/l in the drinking water for 119 h.

Tissue	0 h		26 h		96 h		122 h	
	Range	Mean ±SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Skin	35 - 62	44 ± 13	16 - 26	19 ± 4.3	4.9 - 11.1	7.8 ± 2.5	5.6 - 12.9	8.7 ± 2.8
Muscle	21 - 62	36 ± 16	<LOD		<LOD		NM	
Liver	191 - 929	483 ± 250	5 - 7.5	6.2 ± 0.9	<LOD (5) - <LOQ (1)		NM	
Kidney	112 - 550	229 ± 160	<LOD (4) - <LOQ (2)		<LOD (5) - <LOQ (1)		NM	
Fat	<LOD		<LOD		<LOD		NM	

Values are the range for 6 birds with the mean ± SD; LOD is 2.5 μg/kg and LOQ is 5 μg/kg; NM = not measured.

The residues of parent drug were highest in liver and kidney tissues at zero withdrawal time. The concentration of parent drug fell very rapidly and strongly indicates that this compound is a minor component of the total residues (TR); e.g. (see Table 5) in liver tissues at 18 h mean total residues (TR) = 70 μg/kg, at 36 h mean TR = 21 μg/kg, whereas at 26 h mean sarafloxacin = 6 μg/kg which is probably <20% of TR. In the metabolism study (see above in Table 4) Sarafloxacin formed 65 - 69% of the TR at 6 h. No comparable data was available for kidney but it is most likely that parent drug becomes a minor component of TR at 26 h after drug withdrawal.

The residues of sarafloxacin persisted in the skin. The levels (<13 μg/kg) were low and were not in conflict with the absence of residues in radiodepletion study since they are below the sensitivity of the radio-method (21 μg/kg).

Turkeys

Turkeys weighing 6 - 8.7 kg were given sarafloxacin in their drinking water for 120 hours at a concentration of 21.1 - 28.5 mg/L (equiv. to 2.88 mg/kg BW/day). Groups of six birds were sacrificed at 0, 24 and 120 h after drug withdrawal (Volume 52). Muscle, liver, lung, skin, fat and kidney samples were collected and the concentrations of sarafloxacin measured by HPLC (Volume 60). The results are shown in Table 8.

The residues of parent drug were highest in skin tissues at zero withdrawal time. The concentration of parent drug in liver was low relative to the concentration of TR and strongly indicates that sarafloxacin is a minor component of the total residues, probably about 20% of TR. No comparable data was available for kidney but it is most likely that parent drug is a minor component of TR.

The residues of sarafloxacin persisted in the skin in line with those observed in the radiodepletion study. The levels in both skin and muscle suggest that the parent drug is the major component of residues in these tissues.

Table 8. Residues (μg/kg) in turkeys after administration of sarafloxacin at 21 - 29 ppm in the drinking water for 120 h.

Tissue	0 h		24 h		120 h	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Skin	35 - 62	44 ± 13	16 - 26	19 ± 4.3	4.9 - 11.1	7.8 ± 2.5
Liver	18 - 54	34 ± 16	3 - 7.8	4.5 ± 1.7*	<LOD	
Muscle	4.2 - 5.9	5.3 ± 0.7*	<LOD		<LOD	
Kidney	6 - 19	12 ± 5	<LOD		<LOD	
Fat	<LOD		<LOD		<LOD	

Values are the range for 6 birds with the mean ± SD. LOD is 2.5 μg/kg and LOQ is 5 μg/kg
*Some values were <LOQ but >LOD.

Bound Residues/Bioavailability.

Chicken and turkey liver samples were extracted with acidic and basic acetonitrile and 13% - 18% were non-extractable (Volume 57 & 59). Neither the identity nor the antimicrobial activity of the bound residues was investigated.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The analysis of residues of sarafloxacin is fully documented for edible poultry tissues (Volume 49, Volume 60, Volume 61a, 61b, 61c, 61d). These methods specifically measure the free sarafloxacin but not the sarafloxacin conjugates. The methods consist of extracting homogenised tissue (muscle, liver, kidney and skin with adhering fat) with acetonitrile or in the case of fat with a mixture of acetonitrile and dichloromethane. After drying, the extract is dissolved in the mobile phase (water + phosphoric acid + tetramethylammonium chloride/acetonitrile/N,N-dimethylformamide) and analysed using HPLC with fluorescent detection. The characteristics of the method are detailed for both broilers and turkeys.

The linear range was 5 - 4000 μg/kg and the LOQ for all poultry tissues was claimed as 5 μg/kg. In liver tissue the CV for a concentration of 5 μg/kg was 14% in broilers and 11% in turkeys. The recoveries for poultry muscle, liver, kidney and skin with adhering fat ranged between 57 and 67 % with CVs 1.2 - 15.2%. The recoveries from fat were 93% for broilers and 100% for turkeys. The method has not been tested in a collaborative study with other laboratories but good reproducibility was achieved in the sponsor's laboratory when three different persons used four different HPLC columns of the same type. There was no interference in the chromatograms from admixing monensin, narasin, salinomycin, flumequine, enrofloxacin, difloxacin and danofloxacin (see Volume 49). Interference from the matrix was < 5 μg/kg. The method was suitable for the routine analysis of large numbers of samples per day.

Unfortunately the above method does not measure the residues which are present as sarafloxacin conjugates (see Table 4). They form the majority of the total residues in turkey liver (47 - 57%) but only 8 - 13% of TR in chicken livers. The sponsors have studied the hydrolysis of the conjugates (see summary in Table 9) and each type of conjugate required specific hydrolysis (Volume 59).

Table 9. Effects of different hydrolysis procedures on the stability of Sarafloxacin conjugates.

Residue	Acid Hydrolysis	Alkaline Hydrolysis	Enzyme Hydrolysis
Parent Sarafloxacin (SFX)	No effect	No effect	No effect
SFX-sulfamic acid	Deconjugation	No effect	Not studied
SFX-glucuronide	No effect (?)	No effect (?)	Deconjugation
SFX-sulfamic acid-glucuronide	Deconjugation of sulfamic acid	???	Deconjugation of glucuronide

The hydrolysis appeared to quantitatively release the sulfamic acid but no values were given for the deconjugation performance for the enzyme hydrolysis (Volume 59).

APPRAISAL

Sarafloxacin is a fluoroquinolone antibiotic for use in broiler chickens and turkeys. The dose for chickens from 3 weeks of age is 20 mg/L in water equivalent to 4 mg/kg body weight and for turkeys from 7 weeks of age the dose is 30 mg/L in water equivalent to 4 mg/kg body weight.

The drug is readily absorbed and rapidly cleared by rats, mice, dogs, rabbits, chickens and turkeys. When chickens and turkeys were administered ^{14}C-sarafloxacin hydrochloride by gavage 4 times daily for 5 days, more than 79 - 89% of the dose was excreted in the first six hours post dosing.

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The metabolism of ^{14}C-sarafloxacin was studied in chickens and turkeys. No differences in metabolism were observed between males and females but the metabolic profiles in the liver were different between the two species. The main route of metabolism in poultry liver is the formation of either or both a sulphamic acid conjugate at the N-position in the piperazine ring or a glucuronide with the -COOH group. The unidentified minor metabolites were also conjugates because acid or alkaline hydrolysis of the metabolites yielded parent sarafloxacin. More conjugates were present in the turkey liver (47 - 57%) than chicken liver (8 - 13%). Parent drug formed 21% residues in turkey liver and 67% in chicken liver.

Residue Depletion

All the studies examined the residues in equal numbers of both male and female birds. The analysis of the results indicated that there were no significant differences in the values for males or females; therefore the results for both sexes were combined.

Chickens (Broilers). Male and female chickens were administered 0.54 mg/kg BW ^{14}C-sarafloxacin hydrochloride by gavage four times daily for 5 days. The total dose per day was 2.2 mg per bird (3.4 mg/kg BW/day) which represented 85% of the dose proposed for field use of the drug in drinking water (20 mg/L). Groups of six birds were sacrificed at 6, 18, 36 and 72 h after drug withdrawal. Light muscle, dark muscle, liver, skin with adhering fat and fat samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting. There was no significant difference between the means for both sexes. At 6 h post-dosing the residues in µg/kg sarafloxacin equivalents were: light muscle, 35 ± 8; dark muscle, 28 ± 8; liver, 322 ± 92; Fat, 22 ± 21; skin with fat, 29 ±7. The residues were below the LOD of 22 µg/kg in muscle and fat at later time points. In skin plus fat the residues were 26 ± 11 µg/kg at 18h and less than the LOD (21 µg/kg) at 36h and 72h. In liver the residues were 70 ± 75 µg/kg at 18h, 21 ± 4 µg/kg at 36h and <LOD (15 µg/kg) at 72h. Thus the residues were highest and most persistent in the liver tissue. After one day of drug withdrawal the residues were only measurable in liver and skin. Three days after withdrawal no residues were detected in any of the tissues.

Broilers weighing 1.84 -2.54 kg were given sarafloxacin in their drinking water for 119 hours at a concentration of 15.5 - 18.0 mg/L (equiv. to 2.7 mg/kg BW / day). Groups of six birds were sacrificed at 0, 26, 96 and 122 h after drug withdrawal. Muscle, liver, skin, fat and kidney samples were collected and the concentrations of sarafloxacin measured by HPLC. The mean residues in µg/kg of parent drug were highest in liver, 483 at 0 h, 6 at 26 h and <LOD (2.5 µg/kg) at 96 h. In skin with adhering fat the residues were present at all times. Mean concentrations in skin with adhering fat in µg/kg at 0 h were 44; at 26 h, 19; at 96 h, 8 and at 122 h. The residues were not found in fat and only found at the zero time point for muscle at a mean residue concentration of 36 µg/kg and in kidney, 229 µg/kg.

Turkeys. Turkeys weighing about 2.7 - 3.7 kg were administered 4.25 mg by gavage ^{14}C-sarafloxacin hydrochloride four times daily for five days. The total dose per day was 21 mg per bird (approximately 7 mg/kg BW/day), which is higher than the recommended field dose of 4 mg/kg/day (30 mg/L in drinking water) for turkeys of a similar age. Groups of six birds were slaughtered at 6, 18, 36 and 72 h after drug withdrawal.

Light muscle, dark muscle, liver, skin with adhering fat, fat and kidney samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting. At 6h post treatment the mean residue concentrations in µg/kg sarafloxacin equivalents were: light muscle, 12; dark muscle, 12; liver, 388; fat, 52; skin with fat, 28. The residues were below the limit of detection (LOD = 13 µg/kg) in muscle at 18 h and later time points. In fat the mean residue concentrations were 27 µg/kg at 18 h, and less than the LOD (25 µg/kg) at 36 h and 72 h. In skin plus fat the mean residues were 22 µg/kg at 18 h; 19 µg/kg at 36 h; and 20 µg/kg at 72 h. In liver the mean residues were 87 µg/kg at 18 h; 60 µg/kg at 36 h; and 35 µg/kg at 72 h.

In another study, turkeys weighing 6 - 8,7 kg were given sarafloxacin in their drinking water for 120 hours at a concentration of 21.1 - 28.5 mg/L (equivalent to 2.88 mg/kg BW/day). Groups of six birds were sacrificed at 0, 24 and

Bound Residues/Bioavailability

Chicken and turkey liver samples were extracted with acidic and basic acetonitrile and 13-18% were non-extractable, Neither the identity nor the antimicrobial activity of the bound residues was investigated.

Analytical Method for Sarafloxacin

The analytical methods specifically measure the free sarafloxacin but not the sarafloxacin conjugates, which form the majority of the total residues in turkey liver (47 - 57%) but only 8 - 13% of the total residues in chicken livers. The methods consist of extracting homogenised tissue (muscle, liver, kidney and skin with adhering fat) with an organic solvent, drying, dissolving the extract in the mobile phase and analysis using HPLC with fluorescent detection. The characteristics of the method are detailed for both broilers and turkeys. The linear range was 5 - 4000 µg/kg and the LOQ for all poultry tissues was claimed as 5 µg/kg. The CVs were determined for each tissue from six replicates at six concentrations over the concentration range noted above. In liver tissue the CV for a concentration of 5 µg/kg was 14% in broilers and 11% in turkeys. The recoveries for poultry muscle, liver, kidney and skin with adhering fat ranged between 57 and 67 % with CVs 1.2 - 15.2%. The recoveries from fat were 93% for broilers and 100% for turkeys. The method has not been tested in a collaborative study with other laboratories but good reproducibility was achieved in the sponsor's laboratory when three different persons used four different HPLC columns of the same type. There was no interference in the chromatograms from admixing monensin, narasin, salinomycin, flumequine, enrofloxacin, difloxacin and danofloxacin. Interference from the matrix was less than 5 µg/kg. The method was suitable for the routine analysis of large numbers of samples per day.

Choice of Marker Residue

Parent drug is the clear choice as marker residue (MR) because it is the major residue in all tissues. It is neither necessary nor possible to correlate the values observed for the total residues with those found for the parent drug in the unlabeled residue study. This is because the sponsors have shown that the microbial activities of some of the metabolites (N-acetyl-sarafloxacin, N-formyl-sarafloxacin, 3'-oxo-sarafloxacin and the sulfamic acid conjugate of sarafloxacin) are significantly lower than that of sarafloxacin.

Choice of Target Tissues

The residues in poultry are highest in liver and kidney and persist in skin with adhering fat. The kidney is not normally a target tissue for poultry and therefore the main target tissues should be liver and skin with adhering fat (skin and fat). Because muscle is the major edible tissue for poultry and residues are found in this tissue at short withdrawal times an MRL is set for muscle.

Maximum Residue Limits

The ADI for Sarafloxacin is 0 - 0.3 µg/kg body weight. This permits daily 18 µg per 60 kg person. The following factors are used to set MRLs for chickens and turkeys.

1. Sarafloxacin is the marker residue.
2. The microbiological activity of residues other than parent drug is significantly lower.
3. The LOQ for the analytical methods are 5 µg/kg.
4. As no residues are detectable in poultry muscle at 18 h and beyond, the MRL should equal two times the LOQ.
5. The residues in liver and kidney are higher than residues in muscle, skin and fat.
6. The MRL for chickens are equally applied to turkeys

The Committee recommends MRLs f or chickens and turkeys of 10 µg/kg in muscle, 80 µg/kg in liver, 20 µg/kg in fat and of 80 µg/kg for kidney expressed as parent drug. Using these values for the MRLs, the theoretical maximum daily intake is 16 µg.

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Volume 52. Steady state plasma kinetics and tissue depletion study of Sarafloxacin in turkeys. (Report no.: 021/92/0574, dated 24/APR/1992), 1 - END

Volume 57. Total residue depletion and metabolism studies of ^{14}C-radiolabeled Sarafloxacin hydrochloride using male and female broiler chickens. (Report no.: SC910117, dated 15/APR/ 1992) 1 - END.

Volume 58. Combustion analysis of chicken tissue samples containing ^{14}C-Sarafloxacin hydrochloride originating from Battelle study SC 910117. (Report no.: 56630/19/92, dated 23/APR/ 1992), 1 - END.

Volume 59. Total residue depletion and metabolism studies of ^{14}C-radiolabeled Sarafloxacin hydrochloride using male and female turkeys. (Report no.: SC910116, dated 15/APR/1992) 1 - END.

Volume 60. HPLC method for the assay of Sarafloxacin concentrations in poultry (chickens and turkeys) plasma and tissues. Assay Instruction 830/92/0311, dated 18/APR/ 1992. 18 – 33.

Volume 61a. Assay of supplied blood plasma and tissue specimens for concentrations of Sarafloxacin. (Report no.: IBL 900111, dated 25/APR/1990), 1 - 49.

Volume 61b. Feasibility study of the quantitative determination of Sarafloxacin in plasma and tissue of poultry by means of a HPLC-method. (Report no.: 021/92/0324, dated 12/MAR/ 1992), 50 – 87.

Volume 61c. APPENDIX 1. HPLC determination of sarafloxacin in broiler biological matrices (Appendix to report no.: 021/92/0497 dated 23/APR/ 1992, see also volume 51) 88 – 118.

Volume 61d. APPENDIX 1. HPLC determination of sarafloxacin in turkeys biological matrices (Appendix to report no.: 021/92/0574 dated 24/APR/ 1992, see also volume 52) 119 – 144.

SPECTINOMYCIN

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ADDENDUM
to the spectinomycin residue monograph prepared by the 42nd meeting of the Committee and published in FAO Food and Nutrition Paper 41/6, Rome 1994,

INTRODUCTION

Spectinomycin is an aminocyclitol antibiotic. In veterinary medicine it is used therapeutically for bacterial respiratory and enteric infections. Its broad spectrum bactericidal activity is based on its ability to inhibit protein synthesis in the 30S ribosomal sub-unit of the cell. It is administered to cattle, pigs, sheep and poultry as injectable solutions, orally as aqueous solutions or in feed.

Spectinomycin was reviewed previously by the 42nd Committee in 1994 at which time a full ADI was established (0-40µg/kg) based on a microbiological endpoint. Temporary MRLs were recommended for cattle, pigs and chickens in kidney, liver, muscle, fat and cattle milk as parent drug. The MRLs were designated as temporary because many of the residue depletion data were either from interim progress reports or from pilot studies. At that time the Committee was aware that additional metabolism studies were being conducted to confirm that the microbiologically active portion of the residues in edible tissues was predominantly spectinomycin. The Committee recommended that results of these studies be available for review by 1996.

Sponsors have submitted results of new studies to the Committee for its consideration including:
a) studies in species considered by the 42nd meeting of the Committee;
b) studies to support adding sheep to the list of species;
c) a proposal for an MRL in chicken eggs; and
d) studies to propose adjusted MRL's in muscle and fat.

These new studies provide data on pharmacokinetics in cattle, pigs and sheep; residue data from cattle, pigs, chicken and sheep; and information on assay methodology validation including a comparison between microbiological inhibition and chemical methods.

These new studies provide data on:
a) rapid absorption following IM and SC dosing as well as high bioavailability from these dosing regimens;
b) kidney tissues containing the highest residues of parent drug for long withdrawal periods, while in liver, dihydrospectinomycin is the primary residue that persists for about as long a time as parent drug in kidney;
c) identification of eight metabolites showing that spectinomycin is the major residue in kidney and dihydrospectinomycin is the major metabolite in liver; and
d) concentrations of metabolites in edible tissues indicating muscle and fat have very low concentrations except for skin and underlying fat in chickens.

The new studies also provide additional residue data. They provide information that verifies that:
a) kidney is the target tissue and parent spectinomycin is the marker residue;
b) almost all the microbiological activity in kidney and other edible tissues except liver is accounted for by parent drug; in liver dihydrospectinomycin accounts for most of the microbiological activity; and
c) quantities of microbiologically active residues in other tissues are very low.

The data are most complete for cattle, however, where comparable data are available in other species, it indicates profiles similar to that found in cattle. One sponsor acknowledges that they have not conducted extensive, contemporary species-specific studies to generate data for all species for this old compound. The sponsor suggests the

existing database is satisfactory. Using available data on kidney as the target tissue as an example, they acknowledge that they do not have direct comparisons of the microbiological activity of parent drug in kidney from other species including sheep and pigs. However, data from a radiolabel spectinomycin oral dosing study in pigs indicates very similar absorption characteristics and dose excretion patterns as in cattle, as well as distribution of residues in the tissues. Residue analysis from the pig study indicates that the highest concentrations of spectinomycin residues are in the kidney at all time periods and the highest concentrations of residues in the liver are dihydrospectinomycin. Details are described below.

Pharmacokinetic Data

Cattle

A new pharmacokinetic study of spectinomycin in calves following a single IM, IV and SC injection at a dose of 10 mg spectinomycin/kg BW has been reported using six male Friesian calves about seven weeks old (Caputo, et al., 1995). Each animal was injected once by each route with a washout period of seven days between each treatment. Results are summarized in Table 1. Spectinomycin is rapidly absorbed with IM and SC administration and has a relatively short elimination half-life in calves. It is also completely bioavailable by the IM and SC routes.

Table 1. Pharmacokinetic data using a single dose of 10 mg ^{3}H-spectinomycin/kg BW in calves. by IV, IM and SC administration, respectively

Pharmacokinetic parameter	Intravenous	Intramuscular	Subcutaneous
C_{max}(μg/mL)		27	19.9
t_{max}(h)		0.61	1.06
$AUC_{0-\infty}$ (μg·h/ml)	65.1	76.7	77.3
$T_{1/2}$ (terminal) (h)	1.76	1.52	1.83
T (h)	2.26	2.69	3.04
F (%) (bioavailability)		118	120

Using a full dose disposition study with 16 animals and the highest recommended dose (15 mg ^{3}H-spectinomycin free base equivalents/kg BW) once daily during five consecutive days, data for urine, blood, tissues and feces are summarized in Table 2 (Hornish, et al., 1996a, 1996b). There was a small sex difference accounted for by the method of collection. Greater than 90% of the excreted dose was eliminated within 24 hours after the last dose. The terminal elimination phase of total plasma pharmacokinetic residue had a half-life of about 8 days. The tritiated water accounted for less than 4% of the dose. There appeared to be appreciable retention of drug residues in tissue, primarily in liver and kidney, while muscle and fat retained lesser amounts of total residue (most of which in the latter withdrawal times was accounted for as tritiated water) as shown in Table 3 (Hornish, et al., 1996b). Results are the mean residue concentration in mg/kg (n = 4 animals per group). These data are corrected for tritiated water.

Table 2. Summary of dose accountability of ^{3}H-spectinomycin in cattle receiving five daily subcutaneous doses (% recovered)

Withdrawal Time (days)	Urine	Faeces	Tissues	Total
1	69.35	7.67	3.30	80.31
5	84.48	5.32	1.42	91.23
10	77.07	6.26	0.89	84.21
15	77.17	8.35	0.71	86.22

The spectinomycin related HPLC residue profiles of the urine metabolites were determined in the day 1-5 (on-treatment) urine samples. Eight metabolites were identified by HPLC/APCI (atmospheric pressure chemical ionization) mass spectrometry. Parent drug accounted for approximately 62-64% of the on-treatment urinary residues with all other metabolites being less than 9%. The only major residue identified in kidney was spectinomycin, and in liver the major metabolite was dihydrospectinomycin. For all 16 animals in the study the actual ranges of spectinomycin in kidney were 6.6-15.3% total residues, whereas the maximum amount in liver tissues was less than 4.2% of all residues. The concentration of residues in muscle and fat were too low to allow for meaningful metabolite profiling and identification.

Since the ADI is based on a microbiological endpoint, identification of the metabolites, other than their microbiological activity equivalents is less important.

Table 3. Summary of total residue concentrations (mg/kg) in cattle tissue using a single dose of ^{3}H-spectinomycin subcutaneously daily for five days

Withdrawal Time (days)	Liver	Kidney	Muscle	Fat
1	32.4	59.6	1.03	1.27
5	18.8	14.2	0.36	1.06
10	7.54	4.50	0.36	0.83
15	4.54	2.66	0.29	0.77

Pigs

One new study was reported comparing the plasma pharmacokinetics and bioavailability of spectinomycin sulfate and hydrochloride salts in pigs after a single IM injection of 15 mg spectinomycin free base equivalents/kg BW (Cameron, *et al.*, 1997a). The study used 12 pigs and used a two-week washout period between treatments. Blood samples were collected at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12 and 24 h post treatment. The results are summarized in Table 4 and should be compared to data in Table 1. Available data indicate comparable pharmacokinetic results with cattle.

Table 4. Pharmacokinetic data in pigs after IM injection with 15 mg spectinomycin free base equivalents/kg BW

Pharmacokinetic Parameter	Hydrochloride	Sulfate
$AUC_{0-\infty}$ (µg·h/ml)	88.7	107.6
C_{max} (µg/ml)	43.1	47.7
T_{max} (h)	0.40	0.45

Sheep

The pharmacokinetic data in sheep was generated using a similar design as was used for cattle (Craigmill, *et al.*, 1995a). Ten sheep were treated with a single IV and single and multiple IM injection using a dose of 15 mg/kg (5 mg lincomycin + 10 mg spectinomycin/kg BW. The two injections were separated by a three-week washout period. Blood samples were collected before each injection and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours. Results are summarized in Table 5 and data represent mean values (n = 5 animals per group).

The spectinomycin concentration versus time data following IV dosing were fit to a one compartment open model as were the data following the IM administration (with first order kinetics). Spectinomycin was completely bioavailable after IM dosing. Again the data are comparable to the cattle data in Table 1. After multiple dosing of 15 mg spectinomycin for three consecutive days, there were no significant differences in C_{max} values, AUC and accumulation ratios from dose 1 to dose 3. There was no accumulation following multiple dosing based on the accumulation ratios calculated from C_{max} and C_{min}.

Table 5. Pharmacokinetic parameters of spectinomycin in sheep after a single IV dose and a single IM dose with Linco-Spectin®

Pharmacokinetic Parameter	IV Administration	IM Administration
C_{max} (µg/mL)		23.1
t_{max}	-	0.78
$AUC_{0-\infty}$ (µg-h/mL)	71.2	72.7
$t_{1/2}$ (h)	1.34	1.62
MRT (h)	2.1	2.6
F (%) (bioavailability)	-	104

Residue Data

Cattle

Residue depletion was studied using radiolabeled and unlabeled spectinomycin. The radiolabeled study was the same study used to generate some of the pharmacokinetic data in cattle (Hornish, *et al.*, 1996a, 1996b). For this reason, details will not be repeated other than to indicate that 16 animals were used in the study. Total residues and spectinomycin parent drug were determined with parent drug residues reported using an HPLC method. Results are summarized in Table 6. Results are mean values (n = 4) and concentrations are in mg/kg.

Table 6. Total (parent) spectinomycin residues in bovine kidney and liver after five consecutive daily 15 mg/kg BW subcutaneous doses

Withdrawal Time(days)	Kidney	Liver	Muscle	Fat
1	59.6 (9.12)	32.4 (1.36)	1.03	1.27
5	14.2 (1.74)	18.8 (0.58)	0.36	1.06
10	4.50 (0.42)	7.54 (0.20)	0.36	0.83
15	2.66 (0.20)	4.54 (0.14)	0.29	0.77

The linear regression curves for mean residue concentrations of spectinomycin in kidney and liver are shown below:

- Kidney: $y = -0.5774x + 7.3451$ $r = -0.8310$ y = concentration (mg/kg), x = days
- Liver: $y = -0.0841x + 1.2215$ $r = -0.9096$

Two residue depletion studies were reported using unlabeled spectinomycin. The first residue depletion study was conducted with 24 beef cattle treated subcutaneously once daily for five consecutive days with 15 mg/kg BW unlabeled spectinomycin per day with six animals in each group (Hornish, *et al.*, 1996d). Residues are summarized in Table 7 and reported as mean concentrations in mg/kg. The dose regimen is the sponsor's highest recommended treatment dose in cattle. Residues were determined by the sponsor's proposed HPLC method. Fat did not contain detectable concentrations of parent drug (LOQ = 0.10 mg/kg) at any post treatment time. Liver residues declined to below the LOQ by day 15.

Table 7. Residues of parent spectinomycin in bovine tissue after 5 consecutive daily 15 mg/kg BW subcutaneous doses

Withdrawal Time (days)	Kidney	Liver	Muscle	Inj. Site	Fat
5	3.97	0.28	0.23	0.38	<0.10
10	0.95	0.08	0.15	0.14	<0.10
15	0.27	<0.04	0.13	0.20	Not analyzed
20	0.16	<0.04	0.13	0.20	Not analyzed

The regression equations for the mean residue concentrations of spectinomycin are as follows:

- Kidney: $y = -0.2818x + 5.025$ $r = -0.8604$
- Muscle: $y = -0.0064x + 0.24$ $r = -0.8677$

Because the ADI is based on a microbiological endpoint, a study was reported that evaluated the relationship of a sponsor's microbial inhibition assay for residues in liver and kidney to parent drug (by HPLC) and to total residues (Hornish, *et al.*, 1996c). The tissues used were those from a previous pharmacokinetic study. The microbial inhibition assay was measured by a cylinder plate assay that was not very sensitive to either parent drug or its metabolites. The microbiological inhibition assay was modified from a method used to assay feeds and used *E. coli* #UC527. As used, the assay LOQ was about 4 mg/kg. The LOQ limited the method to analysis of day 1 kidney and day 1 and day 5 liver samples only. The chemical assay was an HPLC method recommended by the sponsor for analysis of spectinomycin residues. For kidney, the ratio of microbiological to parent drug was 0.986 and for liver, the corresponding ratio was 5.61 (day 1) and 3.99 (day 5). This result supports the HPLC method for residue analysis in kidney tissue. Since the only major residue found in liver is dihydrospectinomycin, this metabolite is responsible for almost all the activity

found in liver, even though it is less than 10% of the activity of the parent drug (Salmon, *et al.*, 1994). Results support liver residues as spectinomycin equivalents.

As the above study used almost two year old tissue, a repeat study was conducted with fresh incurred residues from 20 treated and 2 control animals (Hornish, *et al.*, 1997a). Results from residues analyzed from five time frames (1, 2, 3, 5, 10 days) gave a ratio of microbial inhibition versus the HPLC assay of 0.89 to 0.97 for kidney, again supporting the HPLC assay for residue analysis. For liver tissues, only one value was determined because residues beyond one day were below the LOQ of the microbiological inhibition assay. The ratio for day 1 residues in liver was 3.62, consistent with the previous study. Results are summarized in Table 8. The ratios reported in Table 8 are an average of the individual ratios (Hornish, *et al.*, 1997a, 1997b).

Table 8. Comparison of residues determined by HPLC and microbiological assays in cattle tissues following five daily doses at 15 mg/kg SC

Withdrawal Times (days)	Kidney			Liver		
	HPLC (mg/kg)	Micro CP (mg/kg)	Ratio MIC/HPLC	HPLC (mg/kg)	Micro CP (mg/kg)	Ratio MIC/HPLC
1	17.90	17.1	0.95	1.18	3.7	3.62
2	9.42	8.7	0.91	0.67	<0 2	n/a
3	6.75	6.i	0.89	0.54	<0 2	n/a
5	4.34	4.2[a]	0.97	0.55	<0 2	n/a
10	1.09	< LOQ	n/a	0.16	<0 2	n/a

a. One sample result was below the LOQ of 4 mg/kg. One-half the LOQ was used to calculate the mean value for the four samples. See Hornish, 1997a, page 38.

Residues in muscle tissue were determined using the sponsors HPLC method only because residues were below the sensitivity of the microbiological inhibition assay. For the five withdrawal periods noted above, the mean residue concentrations in muscle tissue in mg/kg were 0.42, 0.38, 0.34, 0.26 and 0.12, respectively.

The second residue depletion study with unlabeled spectinomycin was carried out using 20 calves with an average weight of 123 ± 7.1kg. The calves (n = 4 per group) were administered Spectam® G.A. as an injectable solution containing 10% spectinomycin free base (as the dihydrochloride salt) at 30 mg/kg BW spectinomycin daily for five days. Animals were sacrificed at 1, 3, 7, 10 and 14 days post treatment. The results are summarized in Table 9 (Guyonnet, 1995a). Residues in fat were below the limit of quantitation (0.25 mg/kg) at all withdrawal times. For liver and kidney the LOQ was 0. 5 mg/kg and for muscle the LOQ was 0.15 mg/kg.

Table 9. Spectinomycin residues (mg/kg) in calf tissues after five daily doses at 30 mg/kg BW

Withdrawal Time (days)	Kidney	Liver	Muscle	Muscle Inj. Site
1	105.94	6.41	1.15	19.17
3	43.05	4.65	0.67	16.76
7	9.55	1.55	0.36	4.15
10	4.18	1.37	0.25	4.33
14	2.75	0.90	0.20	1.31

Milk

One new study on milk was reported (Guyonnet, 1995b). Eight Holstein cows weighing 638 ± 61 kg, ages 2-8 years with milk production of 30-35 liters (n = 4) and 17-22 liters (n = 4) were treated with Spectam® G.A. at 30 mg/kg BW per day as three 10 mg/kg doses daily for five days. Doses were intramuscular in the neck. The LOQ for milk was

reported as 0.10 mg/L. Residues at 48 hours and at later milkings were below the LOQ. Residues are summarized in Table 10 (n = 8) in mg/L. Residues at 48 hours are for four cows.

Table 10. Residues in milk (mg/L) for dairy cows dosed IM at 30 mg/kg BW for five days

Milking Time (h)	Mean	Minimum/Maximum
12	1.59	0.89-2.11
24	0.45	0.21-0.90
48	0.14	0.13-0.16

Pigs

Three residue studies were reported by the sponsors - one with radiolabeled and two with unlabeled spectinomycin. The radiolabeled study used 20 animals (31.2 ± 7.2 kg) with 88 mg/kg medicated feed (containing 44 mg/kg lincomycin and 44 mg/kg spectinomycin) for seven consecutive days (Jaglan, *et al.*, 1994). This feeding regimen provided an average consumption of 2.73 mg/kg BW per day. Average consumption of the medicated feed was 12.9 ± 1.7 kg/day. Excretion was primarily in the faeces (72.3%) and urine (7.2%). Total residues of ^{3}H-spectinomycin free base in tissues in mg/kg (n = 4 animals per group) are summarized in Table 11. Residue concentrations are corrected for tritiated water. Total residues of spectinomycin in the medicated feed treatment are low, consistent with the poor bioavailability (ca. 10%) by this route of administration.

Table 11. Total residues (mg/kg) of ^{3}H-spectinomycin in pigs after 7 days continuous medicated feed treatment

Withdrawal Time (days)	Kidney	Liver	Muscle	Fat
0 (8 h)	0.64	0.21	0	0.16
1	0.46	0.14	0	0.14
3	0.24	0.10	0	0.17
7	0.06	0.06	0	0.17
10	0.02	0.02	0	0.14

A study using 12 pigs administered unlabeled spectinomycin compared residues in kidney and the injection site after a single IM dose of spectinomycin as the sulfate and hydrochloride salt, each at 15 mg/kg BW (Cameron, et al., 1997b). Mean concentrations of spectinomycin residues by the sponsors HPLC method (LOQ = 0.1 mg/kg) were determined at 1, 2 and 5 days post treatment (n = 4 animals per group). These results are summarized in Table 12.

Table 12. Spectinomycin residues (mg/kg) in pigs following a single dose 15 mg/kg BW IM injection[a]

Withdrawal	Spectinomycin hydrochloride		Spectinomycin sulfate	
Time (days)	Kidney	Injection Site	Kidney	Injection Site
1	9.6	4.8	10.7	3.5
2	6.4	3.4	7.3	1.9
5	1.9	0.8	2.3	0.7

a. Residues at the injection site deplete to <0.3 mg/kg by day 5.

The second study was in 20 piglets (4.82 ± 1.13kg, 16 days old) using an oral solution containing 5% spectinomycin free base (as the dihydrochloride salt) with a pump delivery dose of 1 ml containing 50 mg spectinomycin (Guyonnet, 1996). This equates to mean doses of 29.1 mg/kg BW every 12 hours at the beginning of the trial and 25.0 mg/kg BW

every 12 hours at the end of the five day dosing study. Mean residues (mg/kg) are summarized in Table 13 using the sponsor's HPLC method (LOQ = 0.5 mg/kg in kidney and liver; 0.25 mg/kg for fat/skin; and 0.3 mg/kg for muscle).

Table 13. Spectinomycin residues (mg/kg) in piglets dosed orally for five days at 25-29 mg/kg BW

Withdrawal time, days	Kidney	Liver	Muscle	Skin/Fat
1	18.15	2.15	0.64	0.69
3	7.64	1.03	<0.3	0.39
7	4.41	0.84	<0.3	<0.25
10	1.90	<0.5	<0.3	<0.25
14	<0.5	<0.5	<0.3	<0.25

Chickens

Two residue depletion studies were reported. In one study using 7-8 week old broiler chickens (84 chickens, BW = 1412-2057 g) were dosed with Linco-Spectin® soluble powder (100 mg spectinomycin and 50 mg lincomycin per kg BW) in the drinking water (Cameron, *et al.*, 1996, Nappier, et al., undated). Groups of 12 birds were sacrificed at 0, 6, and 12 hours, 1, 2, 4 and 8 days post treatment. Each data point is a two bird composite (n = 6 sampling units per sampling time). Results are reported in mg/kg as mean values. The authors noted that there was an interfering peak in analyzing liver samples for spectinomycin using the proposed HPLC method (LOQ = 0.1 mg/kg). Residues in liver are reported using a modified version of the original method (Nappier, et al., undated). Results are summarized in Table 14.

Table 14 Residues of spectinomycin (mg/kg) in broiler chickens after oral administration of 100 mg/kg BW spectinomycin and 50 mg/kg BW lincomycin for seven days

Withdrawal time	Kidney	Liver	Muscle	Skin plus underlying fat
0 hour	2.0	0.43	0.5	2.9
6 hour	4.2	0.38	0.3	1.7
12 hour	1.0	0.27	0.3	1.3
1 day	0.6	0.22	0.1	0.7
2 days	0.7	0.12	0.1	0.5
4 days	<0.1	<0.1	<0.1	0.2
8 days	<0.1	<0.1	<0.1	0.3

The second residue study used 36 broiler chickens (859-1255 g, 44 days old) treated five days with 50 mg/kg BW of spectinomycin (Guyonnet, 1997). The drug was administered as an oral powder containing 50% spectinomycin, mixed into the drinking water. The birds were sacrificed at days 1, 4, 7, 11 and 14 post treatment. No spectinomycin residues could be quantified in any sample at any of the withdrawal times based on the sponsor's LOQ of 0.5 mg/kg in liver and kidney; 0.3 mg/kg in muscle and 0.25 mg/kg in fat.

These two data sets show relatively lower amounts of residues in edible tissues compared with residues from subcutaneous treatments, consistent with oral dosing in other food animal species.

Eggs

No new data were reported for eggs, however, a study was referenced in the 42nd meeting of the Committee (Keppens and DeSutter, 1992). In that study, birds received four different treatments for seven days. Three treatments were 1:1 mixtures of spectinomycin-lincomycin at 440, 330 and 220 mg/kg in feed. The fourth treatment was 0.5 g/L (0.333 g/L spectinomycin and 0.167 g/L lincomycin) in water. No residues of spectinomycin were detected in eggs from any treatment group either the last two days of treatment or three consecutive days following withdrawal of drug. The sensitivity (limit of determination) of the microbiological method was 2 mg/L.

Sheep

Two new studies were reported in sheep. One new study (Craigmill, *et al.*, 1995b) using twenty crossbred sheep (57-87.5 kg) that were injected IM once daily for three consecutive days at the recommended dose level of 15 mg/kg BW (10 mg/kg spectinomycin and 5 mg/kg lincomycin). Groups of five sheep were sacrificed at 8 hours, 7, 14 and 21 days post treatment. Results (mg/kg) are summarized as mean values in Table 15. The detection limit of the HPLC method was 0.04 mg/kg.

Table 15. Tissue residues of spectinomycin (mg/kg) in sheep following multiple IM injections at 15 mg/kg BW (10 mg/kg BWspectinomycin and 5 mg/kg BW lincomycin)

Withdrawal Time	Kidney	Liver	Muscle	Fat	Injection Site
8 hours	11.99	0.63	0.29	0.19	4.56
7 days	0.51	0.10	<0.04	<0.04	0.08
14 days	0.10	0.08	<0.04	<0.04	<0.04
21 days	<0.04	<0.04	<0.04	<0.04	<0.04

The linear regression curve for mean residues of spectinomycin in kidney is shown below:

$$\text{Kidney: } y = -0.5166x + 8.5542 \quad r = -0.7746$$

The second study employed 24 sheep (43.9 ± 3.4 kg, ages 6-7 month) treated with 30 mg/kg spectinomycin BW two times per day by IM injection for five days (Guyonnet, 1995c). Withdrawal times were 1, 3, 7, 10, 14 and 18 days. Mean residue concentrations (n = 4 animals per group) are summarized in Table 16. For this study, the LOQs are 0.5 mg/kg in liver and kidney; 0.15 mg/kg for muscle; and 0.25 mg/kg for fat.

Table 16. Spectinomycin residues (mg/kg) in sheep following IM injections of 30 mg/kg BW twice daily for 5 days

Withdrawal Time (days)	Kidney	Liver	Muscle	Muscle (Inj. Site)	Fat
1	99.96	4.78	0.43	16.30	0.41
3	47.42	3.18	0.25	4.09	<0.25
7	10.31	1.24	<0.15	2.25	<0.25
10	3.89	0.90	<0.15	0.86	<0.25
14	1.75	0.83	<0.15	0.46	<0.25
18	0.78	<0.5	<0.15	0.17	<0.25

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

One sponsor has developed and reported on the performance assessment and validation of their quantitative method for determining residues of spectinomycin in food animal tissue (Jaglan and Haagsma, 1993, Haagsma, 1995, Weist and Hornish, 1995a). The method involves isolation involving solvent extraction and solid phase extraction clean-up using a citrate buffer solution followed by HPLC analysis. The HPLC procedure employs a one-column gradient elution procedure with post column oxidation using hypochlorite solution and derivatization with o-phthalaldehyde to form fluorescent derivatives for detection of spectinomycin. The method was evaluated for potential interferences with seven other antibiotics: ceftiofur, erythromycin, lincomycin, neomycin, penicillin G, sulfadimethoxine and tylosin. No interferences were noted.

Results of the method validation (concentration limits of 0.10-10.0 mg/kg) demonstrated that there was acceptable day-to-day variability, acceptable recoveries in all tissues, and the method has a limit of quantification (LOQ) of 0.10 mg/kg. Specifically, in kidney the recoveries were 81-87% with a Coefficient of Variation (CV) of 6.2%; in liver, recoveries were 85-90%; in muscle and fat, recoveries were 89-93%. For kidney, the target tissue, the LOQ was validated at 0.1 mg/kg with a recovery of 87.2 ± 17.0% (CV=19.5%). Day-to-day variability in kidney was from 2.2-

9.8% at 2.5-10.0 mg/kg. The quantitative performance was successfully compared in bovine kidney samples obtained from fortified control tissue and from biologically incurred residue tissue. Two laboratories were involved in the method performance studies. The Haagsma-based HPLC method (Weist and Hornish, 1995a) was compared with the original method developed by Haagsma (Jaglan and Haagsma, 1993, Haagsma, 1995) and found to be less repeatable over different days, but both methods give analytical results that were not statistically different. The sponsors concluded that with 95% confidence, the two methods provided the same average analytical results for spectinomycin in bovine kidney within 5.6% ± 4.6% (Hornish, *et al.*, 1996e). A set of 12 samples can be processed in 6-8 hours.

The sponsor for the method described above noted the HPLC method is applicable to and has been validated for all tissues, however, the potential exists in some liver samples for interference from dihydrospectinomycin, the major metabolite, and an endogenous liver component. To address this potential interference, a minor modification in the chromatography is suggested (Hornish and Weist, 1997b). The modification for liver samples employs a reverse phase separation rather than an ion-exchange separation to completely resolve parent spectinomycin from the interferences for accurate quantification. The sample preparation and the HPLC post-column detection system remained the same for all tissues.

The second sponsor also has reported an HPLC method as well for spectinomycin (Guyonnet, 1995a, p38, *et seq.*). The procedure involves homogenization, followed by liquid-liquid extraction, elution using a C_{18}-solid phase extraction cartridge, derivatization with 2,4-diphenylhydrazine, and quantification by HPLC on a C_{18}-column using ultraviolet detection at 405 nm (except in chicken tissue where 465 nm was used). Performance was determined by an intralaboratory one analyst procedure. Although no ruggedness testing was indicated, interference was checked against lincomycin, gentamicin and trospectinomycin. For kidney and liver, performance testing was evaluated using fortified tissues at 0.5-20 mg/kg; in muscle, 0.15-5.0 mg/kg; in fat, 0.25-10 mg/kg and in milk, 0.1-10 mg/L. Recoveries in muscle, kidney and liver were 72-80%; in milk, 85-92%; and in fat, 80-90%. The limit of quantification in kidney and liver was 0.5 mg/kg; in fat, 0.25 mg/kg; in milk, 0.1 mg/L; in cattle and sheep muscle, 0.15 mg/kg; in chicken muscle, 0.25 mg/kg; and in pig muscle, 0.3 mg/kg. The LOQ's were determined using a CV of 15%. Though the method was indicated as being suitable as a regulatory method for routine analysis, there was no indication of the number of samples that could be analyzed per day.

A confirmatory method was also reported by one sponsor (Wiest and Hornish, 1995b). It is a two-dimensional method that employs HPLC and atmospheric pressure chemical ionization (APCI) collision induced dissociation (CID) mass spectrometry (MS/MS). Three criteria were met for confirmation: the signal-to-noise ratio for four reaction product ions at m/z 98, 116, 158 and 189 (derived from the m/z 333 for the protonated ion of spectinomycin) were greater than 3-to-1; an HPLC retention time of spectinomycin in the tissue sample within ± 2 minutes relative to an external spectinomycin standard; and a relative abundance of four reaction products ions in the sample within ±10% relative to the external standard. The method was shown to confirm spectinomycin residues to a lower limit concentration of 0.05 mg/kg. Routine confirmation for bovine kidney is 0.1-10 mg/kg, and for other tissues, concentrations of 0.05-1 mg/kg.

APPRAISAL

Spectinomycin was reviewed previously by the 42nd meeting of the Committee at which time a full ADI was established (0-40 µg/kg) based on a microbiological endpoint. Temporary MRL's were recommended for cattle, pig and chickens in kidney, liver, muscle, fat, and cattle milk as parent drug. The MRL's were designated as temporary because many of the residue depletion data were either from interim progress reports or from pilot studies.

The two sponsors have submitted new studies that provide data on pharmacokinetics in cattle, pigs and sheep; residue depletion data for cattle, pigs, chickens, sheep, and milk in cattle; studies to support adding MRL's in sheep; and studies to propose new MRL's in muscle and fat. Information was provided on two residue methods for quantification, including validation data on one of the quantitative methods, and a confirmatory method. A study comparing the microbiological inhibition and chemical assay methods also was provided.

Pharmacokinetic Studies

Pharmacokinetic studies in cattle and sheep using radiolabeled spectinomycin at 10 mg/kg BW as an injectable solution show nearly identical pharmacokinetic parameters, including rapid uptake of the drug and complete bioavailability. There was little difference between intramuscular or subcutaneous treatments. In a full disposition study in cattle given 15 mg/kg BW radiolabeled spectinomycin subcutaneously daily for five days, more than 90 percent of the excreted radioactivity was eliminated within 24 hours after the last dose. The terminal elimination phase of plasma residues had a half-life of about 8 days. Residues from urine, faeces and tissues accounted for 80-91% of the dose with urine accounting for 69-84% of the total, faeces 5-8% and tissues 1-3%. The residues in tissue for 1-15 days post treatment

are highest in kidney and liver, with muscle and fat having much lower amounts. Approximately 85-90% of the residues are found in liver and kidney.

Cattle Eight spectinomycin metabolites were identified by HPLC/mass spectrometry in the urine from a radiolabeled calf study using 16 animals. Parent drug accounted for 62-64% of the on-treatment urinary residues with all other metabolites being less than 9% each. The major residue identified in kidney was spectinomycin, and in liver the major metabolite was dihydrospectinomycin. The actual amounts of spectinomycin in kidney were 6.6-15.3% of the total residues, whereas the maximum amount in liver tissues was less than 4.2% of all residues. The concentration of residues in muscle and fat were too low to allow meaningful metabolite profiling and identification. Since the ADI is based on a microbiological endpoint, identification of the metabolites other than their microbiological activity equivalents is less important. These studies support the studies reviewed at the 42nd meeting of the Committee, identifying the kidney as the target tissue and spectinomycin as the marker residue.

Pigs Pharmacokinetic studies in pigs given 15 mg/kg BW spectinomycin free base equivalents as either the hydrochloride or sulfate salt by intramuscular injection gave comparable but somewhat higher area-under-the-curve values and shorter times to maximum concentration in plasma than in cattle indicating a more rapid absorption in pigs.

A radiolabeled study in pigs using 88 mg/kg medicated feed (1:1 ratio of lincomycin and spectinomycin) for seven days was reported. This feeding regimen provided an average daily consumption of 2.73 mg/kg BW. Excretion of radioactivity was mainly in the faeces (72.3%) and urine (7.2%). Total residues in kidney at day 1 were 0.46 mg/kg and residues declined to 0.02 mg/kg on day 10. In liver tissue, the total residues at day 1 were 0.21 mg/kg and declined to 0.02 mg/kg at day 10. No residues were detectable in muscle tissue after correcting for tritiated water. Values in fat remained constant between days 1-10 at about 0.15 mg/kg. Total residues were low, consistent with the poor bioavailability by this route of administration.

Sheep The pharmacokinetic data in sheep were generated using a design that was used for cattle. Pharmacokinetic parameters were nearly identical by either subcutaneous or intramuscular injection using a dose of 15 mg/kg BW. Spectinomycin was bioavailable and after single doses for three consecutive days there was no significant difference in the pharmacokinetic parameters measured and also no indication of accumulation of residues from day 1 to day 3.

Residue Depletion Studies

Cattle Three residue depletion studies in cattle were reported - one using radiolabeled drug and two with unlabeled spectinomycin. In the radiolabeled (s.c.) study using 15 mg/kg BW, residues were determined at days 1, 5, 10 and 15 post treatment. Total residues in kidney depleted from 59.6 mg/kg on day 1 to 2.66 mg/kg on day 15. Spectinomycin residues in kidney by the HPLC method [limit of quantification (LOQ) was 0.1 mg/kg] were 9.12 mg/kg and 0.20 mg/kg on day 1 and 15, respectively. For liver, total residues on day 1 were 32.8 mg/kg and declined to 4.54 mg/kg on day 15. Spectinomycin residues were 1.36 mg/kg on day 1 and 0.14 mg/kg on day 15. Only total residues in muscle and fat could be determined. In muscle, total residues on day 1 were 1.03 mg/kg and on day 15 they were 0.29 mg/kg. The corresponding total residues in fat were 1.27 mg/kg on day 1 and 0.77 mg/kg on day 15. The unlabeled spectinomycin study using 24 animals treated with five consecutive daily subcutaneous doses of 15 mg/kg BW gave similar amounts of spectinomycin residues in all four tissues. The second study using 30 mg/kg BW unlabeled spectinomycin per day in calves by injection for five days gave higher residues. In kidney, spectinomycin residues on day 1 were 106 mg/kg, declining to 2.75 mg/kg on day 14. In liver, residues on day 1 and day 14 were 6.41 mg/kg and 0.90 mg/kg, respectively. For muscle, residues on day 1 and day 14 were 1.15 mg/kg and 0.20 mg/kg, respectively; residues in fat were below the limit of quantification.

Because the ADI is based on a microbiological endpoint, one sponsor reported two studies that evaluated the relationship of the microbiological inhibition assay with their HPLC method in liver and kidney. The microbiological analysis used a cylinder plate assay that was not very sensitive to spectinomycin (LOQ was 4 mg/kg), while the HPLC assay had a reported limit of quantitation of 0.1 mg/kg. For kidney the ratio of the microbiological assay value to the HPLC assay was approximately 0.98; for liver the ratio was approximately 3.6. This result supports the HPLC method for residue analysis in kidney tissue. Since the only microbiologically active residue in liver is dihydrospectinomycin, this metabolite is responsible for almost all the activity found in liver, even though it is less than 10% of the microbiological activity of parent drug.

One new study in lactating cows using three 10 mg/kg BW spectinomycin injections per day for five days confirmed study results from the 42nd meeting of the Committee. Residues depleted to below the limit of quantification (0.10 mg/L) at 48 hour post treatment milkings.

Two residue studies were reported in pigs using unlabeled drug. In one study using 12 pigs residues were determined following a single intramuscular injection of 15 mg/kg BW. of spectinomyein hydrochloride and spectinomycin sulfate. Residues using the hydrochloride salt on day 1 in kidney were 9.6 mg/kg and declined to 1.9 mg/kg on day 5; at the injection site residues declined from 4.8 mg/kg on day 1 to 0.8 mg/kg on day 5. With the sulfate salt, corresponding residues in kidney on day 1 were 10.7 mg/kg and declined to 2.3 mg/kg on day 5; injection site residues on day 1 and day 5 were 3.5 mg/kg and 0.7 mg/kg. The second study used an oral solution of spectinomycin that ranged from 29-25 mg/kg BW twice daily for five days. Residues in kidney on day 1 were 18.15 mg/kg and declined to less than 0.5 mg/kg on day 14. In liver the residues on day 1 were 2.15 mg/kg and declined to less than 0.5 mg/kg on day 10. Residues in muscle tissue were 0.64 mg/kg on day 1 and less than 0.3 mg/kg on day 3. For fat, residues were 0.69 mg/kg on day 1 and less than 0.25 mg/kg on day 7.

One residue depletion study was reported using 7-8 week old broiler chickens using an oral solution of 100 mg spectinomycin and 50 mg lincomycin per kg BW in the drinking water for seven days. Residues in kidney declined from 2.0 mg/kg at time of withdrawal (0 h) to less than 0.1 mg/kg on day 4. Corresponding residues in liver declined from 0.43 mg/kg at 0 h to less than 0.1 mg/kg on day 4. Residues in muscle were almost identical to residues in liver at all time points. Residues in skin and adhering fat were 2.9 mg/kg at the time of withdrawal, declining to 0.3 mg/kg on day 8. In the second residue study, broiler chickens were treated five days with 50 mg/kg BW of spectinomycin. No spectinomycin residues could be quantified in any sample using the sponsor's method (limit of quantification was 0.5 mg/kg in kidney and liver, 0.3 mg/kg in muscle and 0.25 mg/kg in fat) on day 1 withdrawal. These residue amounts are consistent with other food animal species with relatively low residues from oral administration of spectinomycin.

No new studies were reported for eggs, however, a study was reviewed by the 42nd meeting of the Committee. In that study, laying birds were treated with four different doses of 1:1 mixture of spectinomycin and lincomycin at 440, 330 and 220 mg/kg in feed and a drinking water treatment using a 2:1 mixture of spectinomycin and lincomycin at 0.5 g/L. No residues were detected in eggs in any group during the last two days of treatment and three consecutive days following withdrawal. Residues were assayed using a method with a limit of quantification of 2 mg/kg.

In sheep, two new studies were reported. In the first study, animals were treated for three days by intramuscular injection at 15 mg/kg BW per day using a 2:1 mixture of spectinomycin and lincomycin. Residues in kidney declined from 12.0 mg/kg at 8 hours withdrawal to 0.1 mg/kg on day 14. In liver, residues declined from 0.63 mg/kg at 8 hours to 0.08 mg/kg on day 14. Residues in muscle were 0.29 mg/kg at 8 hours withdrawal and less than 0.04 mg/kg on day 7 withdrawal. Residues in fat were 0.19 mg/kg at 8 hours withdrawal and less than 0.04 mg/kg on 7 days withdrawal. The second study treated the animals with 30 mg/kg BW of spectinomycin two times per day by intramuscular injection for five days. Residues in kidney were 100 mg/kg on day 1 and declined to 0.78 mg/kg on day 18. Corresponding residues in liver were 4.78 mg/kg on day 1 and less than 0.5 mg/kg on day 18. For muscle, residues declined from 0.43 mg/kg on day 1 to less than 0.15 mg/kg on day 7. In fat residues were 0.41 mg/kg on day 1 and less than 0.25 mg/kg on day 3.

Analytical Methods

One sponsor reported results of the method performance trials for two quantitative and one confirmatory method. The preferred quantitative method from one sponsor involves solvent extraction and solid phase extraction followed by an HPLC procedure employing a gradient elution for separation with post column oxidation and derivatization to allow fluorescence detection. The method did not show any potential interference with seven other antibiotics examined. The method was performance tested in two laboratories using fortified and incurred residue samples over a concentration range of 0.1-10 mg/kg in kidney, 0.1-5 mg/kg in liver, and 0.1-1.0 mg/kg in muscle and fat. Performance values for recovery, day-to-day variability and limit of quantification were evaluated. Recoveries in all tissues were greater than 80%. The Coefficient of Variation for all tissues and species evaluated were less than 20%, even at the limit of quantification of 0.1 mg/kg. A set of 12 samples can be analyzed in 6-8 hours. The second method developed by this sponsor was not as reliable as the above method; however, with 95% confidence the two methods provided the same average analytical results for spectinomycin in bovine kidney within 5.6%. This sponsor's confirmatory method was based on atmospheric pressure chemical ionization (APCI) collision induced dissociation (CID) mass spectrometry. Criteria for confirmation included satisfactory ratios for four product ions, HPLC retention time and relative abundance of the reaction product ions compared to an external standard of spectinomycin. These methods are satisfactory for residue analysis.

The second sponsor also reported an HPLC method for quantification of spectinomycin residues using a similar extraction and isolation procedure, derivatization and quantification by HPLC using ultraviolet detection. Performance in all tissues and species noted above was determined in one laboratory. Although no study was reported on optimizing performance of the method, no interference was noted when evaluated with three other antimicrobial drugs. Recoveries in all species and tissues as well as Coefficients of Variation were satisfactory for routine residue analysis, however, the limits of quantification were higher than with the HPLC method noted above. There was no indication of the number of

samples that could be analyzed in one day. The method may be suitable for routine analysis depending on the analytical equipment available.

Maximum Residue Limits

The new pharmacokinetic and residue depletion studies in cattle, pigs, sheep and chickens verify data from the 42nd meeting of the Committee, indicating that kidney is the target tissue and spectinomycin is the marker residue. However, considering the practical limitations of collecting kidney tissue from chickens for residue analysis, skin/adhering fat may be the more appropriate target tissue in chickens.

In reaching its decision on MRLs, the Committee took into account the following information:

- Based on the ADI of 0-40 µg/kg BW for spectinomycin that was established at the 42nd meeting of the Committee, the allowable daily intake of spectinomycin is 2400 µg for a 60-kg person.
- The ADI is based on a microbiological end point.
- The only microbiologically active residues are parent drug and dihydrospectinomycin.
- Parent drug is the only microbiologically active residue in muscle, kidney and fat.
- Dihydrospectinomycin is the major microbiologically active residue in liver.
- The ratio of microbiological activity in liver compared to HPLC-determined spectinomycin is approximately four to one.
- Dihydrospectinomycin microbiological activity is approximately 10% of parent drug.

The Committee recommended the following MRL's:

For cattle, sheep, pigs and chickens: muscle, 500 µg/kg; liver, 2000 µg/kg; kidney, 5000 µg/kg; and fat, 2000 µg/kg. The recommended MRL for cattle milk is 200 µg/L and for eggs, 2000 µg/kg. Residues are expressed as parent drug.

Using these values, the theoretical maximum daily intake of spectinomycin residues is 1800 µg.

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ANNEX 1

SUMMARY OF JECFA EVALUATIONS OF VETERINARY DRUG RESIDUES FROM THE 32ND MEETING TO THE PRESENT

This attached table summarizes the veterinary drug evaluations conducted by JECFA at the 32nd (1987), 34th (1989), 36th (1990), 38th (1991), 40th (1992), 42nd (1994), 43rd (1994), 45th (1995), 47th (1996), 48th (1997) and 50th (1998) meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in food. Please see reports of those meetings, published in WHO Technical Report Series (TRS).

Some notes regarding the Table:

- The "Status" column refers to the ADI and indicates if "No" ADI was established, if a full ADI was given, or if the ADI is Temporary (T).
- Where an MRL is temporary, it is so indicated by "T".
- Several compounds have been evaluated more than once. The data given is for the most recent evaluation.

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Abamectin	0-1 (1995 JMPR)	Full	47 (1996)	100 50	Liver, fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	0-50	Full	34 (1989)	100 5000	Muscle, fat, milk Liver, kidney	Cattle, sheep	MRLs analysed as 2-amino-benzimidazole and expressed as parent drug equivalents, see WHO TRS 788
Azaperone	0-6	Full	50 (1998)	60 100	Muscle, fat Liver, kidney	Pigs	Sum of azaperone and azaperol
Benzylpenicillin	30 µg/person/day	Full	36 (1990)	50 4	Muscle, liver, kidney Milk	All species	Parent drug
BST	Not specified	Full	50 (1998)	Not specified	Muscle, liver, kidney, fat, milk	Cattle	
Carazolol	0-0.1	Full	43 (1994)	5 25	Muscle, fat/skin Liver, kidney	Pigs	Parent drug. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI which is based on the acute pharmacological effect of carazolol
Carbadox	Limited acceptance	Full	36 (1990)	30 5	Liver Muscle	Pigs	Quinoxaline-2-carboxylic acid

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Ceftiofur	0-50	Full	45 (1995) 48 (1997)	 1000 2000 6000 2000 100 µg/l	 Muscle Liver Kidney Fat Milk	 Cattle, pigs Cattle	Desfuroylceftiofur
Chloramphenicol	No ADI		42 (1994)	No MRL			
Chlorpromazine	No ADI		38 (1991)	No MRL			
Chlortetracycline, oxytetracycline, tetracycline	0-30 (Group ADI)	Full	50 (1998)	200 600 1200 400 100 µg/l 100 T 100	Muscle Liver Kidney Eggs Milk Muscle Muscle	Cattle, pigs, sheep, poultry Poultry Cattle, sheep Fish Giant prawn	Parent drugs, singly or in combination Oxytetracycline only Oxytetracycline only
Clenbuterol	0-0.004	Full	47 (1996)	0.2 0.6 0.05 µg/l	Muscle, fat Liver, kidney Milk	Cattle, horses Cattle	Parent drug
Closantel	0-30	Full	36 (1990) 40 (1992)	 1000 3000 1500 5000 2000	 Muscle, liver Kidney, fat Muscle, liver Kidney Fat	 Cattle Sheep	Parent drug
Cyfluthrin	0-20	Full	48 (1997)	20 200 40 µg/l	Muscle, liver, kidney Fat Milk	Cattle	Parent drug
Cypermethrin	0-50	Full	47 (1996)	200 T 1000 T 100 T 50 µg/l T	Muscle, liver, kidney Fat Eggs Milk	Cattle, sheep, chickens Chickens Cattle	Parent drug
α-Cypermethrin	0-20	Full	47 (1996)	100 T 500 T 50 T	Muscle, liver, kidney Fat Eggs	Cattle, sheep, chickens Chickens	

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				25 µg/l T	Milk	Cattle	
Danofloxacin	0-20	Full	48 (1997)	200 400 100 100 50 200 100	Muscle Liver, kidney Fat Muscle Liver Kidney Fat	Cattle, chickens Pigs	Parent drug For ehiekens fat/skin in normal proportion
Dexamethasone	0-0.015	Full	42 (1994) 48 (1997) 50 (1998)	 No MRL No MRL			Temporary MRLs were not extended Regulatory method not available
Diclazuril	0-30	Full	50 (1998)	500 3000 2000 1000	Muscle Liver Kidney Fat	Sheep, rabbits, poultry	Parent drug Poultry skin/fat
Dihydrostrepto-mycin, streptomycin	0-50 (Group ADI)	Full	48 (1997)	500 T 1000 T 200 µg/l T	Muscle, liver, fat Kidney Milk	Cattle, pigs, sheep, chickens Cattle	Sum of dihydrostreptomycin and streptomycin
Dimetridazole	No ADI		34 (1989)	No MRL			
Diminazene	0-100	Full	42 (1994)	500 12000 6000 150 µg/l	Muscle Liver Kidney Milk	Cattle	Parent drug
Doramectin	0-0.5	Full	45 (1995)	10 100 30 150	Muscle Liver Kidney Fat	Cattle	Parent drug. The Committee noted the high concentration of residues at the injeetion site over a 35-day period after subcutaneous or intramuscular administration of the drug at the recommended dose.
Enrofloxacin	0-2	Full	48 (1997)	No MRL			
Eprinomectin	0-10	Full	50 (1998)	100 2000 300	Muscle Liver Kidney	Cattle	Eprinomectin B_{1a}

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				250 20	Fat Milk		
Estradiol-17β	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Febantel, fenbendazole, Oxfendazole	0-7 (Group ADI)	Full	50 (1998)	100 500 100 µg/l	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs, horses, goats Cattle, sheep	Sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents
Fenbendazole (see febantel)							
Fluazuron	0-40	Full	48 (1997)	200 500 7000	Muscle Liver, kidney Fat	Cattle	Parent drug
Flubendazole	0-12	Full	40 (1992)	10 200 500 400	Muscle, liver Muscle Liver Eggs	Pigs Poultry	Parent drug
Flumequine	0-30	Full	48 (1997)	500 1000 3000 500 T 1000 T 3000 T 500 T	Muscle Liver, fat Kidney Muscle Liver, fat Kidney Muscle/skin	Cattle Pigs, sheep, chickens Trout	Parent drug Muscle/skin in normal proportion
Furazolidone	No ADI		40 (1992)	No MRL			
Gentamicin	0-20	Full	50 (1998)	100T 2000 5000 200 µg/l	Muscle, fat Liver Kidney Milk	Cattle, pigs Cattle	Parent drug
Imidocarb	0-10	Full	50 (1998)	300 T 2000 T 1500 T 50 T 50 µg/l T	Muscle Liver Kidney Fat Milk	Cattle	Parent drug
Ipronidazole	No ADI		34 (1989)	No MRL			
Isometamidium	0-100	Full	40 (1992)	100	Muscle, fat, milk	Cattle	Parent drug

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				500 1000	Liver Kidney		
Ivermectin	0-1	Full	40 (1992)	100 40 15 20	Liver Fat Liver Fat	Cattle Pigs, sheep	H_2B_{1a}
Levamisole	0-6	Full	42 (1994)	10 100	Muscle. kidney, fat Liver	Cattle, sheep, pigs, poultry	Parent drug
Metronidazole	No ADI		34 (1989)	No MRL			
Moxidectin	0-2	Full	45 (1995) 50 (1998) 47 (1996) 48 (1997)	20 100 50 500 20 100 50 500 50 20	Muscle Liver Kidney Fat Muscle Liver Kidney Fat Muscle Muscle	Cattle Cattle, sheep Deer Sheep Cattle	Parent drug. The Committee noted the very high concentration and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.
Neomycin	0-60	Full	47 (1996)	500 10000 500 500 µg/l	Muscle, liver, fat Kidney Eggs Milk	Cattle, chickens, ducks, goats, pigs, sheep, turkeys Chickens Cattle	Parent drug
Nicarbazin	0-400	Full	50 (1998)	200	Muscle, liver, kidney, fat/skin	Chicken (broilers)	
Nitrofurazone	No ADI		40 (1992)	No MRL			
Olaquindox	Limited acceptance	T	42 (1994)	No MRL (see remarks)	Muscle	Pigs	MQCA. The Committee recommended no MRLs but noted that 4 µg/kg of MQCA (T) is consistent with Good Veterinary Practice
Oxfendazole (see febantel)							

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Oxolinic acid	No ADI		43 (1994)	No MRL			
Oxytetracycline (see chlortetracycline)							
Procaine benzylpenicillin	Less than 30 µg of penicillin per person per day	Full	50 (1998)	50 4	Muscle, liver, kidney Milk	Cattle, pigs, chickens Cattle	Benzylpenicillin
Progesterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Propionyl-promazine	No ADI		38 (1991)	No MRL			
Ractopamine	No ADI		40 (1992)	No MRL			
Ronidazole	Withdrawn		42 (1994)	No MRL			
Sarafloxacin		Full	50 (1998)	500 2000 5000 2000 200 µg/l	Muscle Liver, fat Kidney Eggs Milk	Cattle, pigs, sheep, chickens Chickens Cattle	Parent drug
Spectinomycin	0-40	Full	42 (1994) 50 (1998)	 500 2000 5000 2000 2000 200 µg/l	 Muscle Liver Kidney Fat Eggs Milk	Cattle, pigs, sheep, chickens Chickens Cattle	Parent drug
Spiramycin	0-50	Full	43 (1994) 47 (1996) 48 (1997)	 200 200 600 600 300 300 800 300 300 200 µg/l	 Muscle Muscle Liver Liver Kidney Kidney Kidney Fat Fat Milk	 Cattle, chickens Pigs Cattle, chickens Pigs Cattle Pigs Chickens Cattle, chickens Pigs Cattle	For cattle and chickens MRLs are expressed as the sum of spiramycin and neospiramycin For pigs MRLs expressed as spiramycin equivalents (antimicrobially active residues)
Streptomycin (see dihydrostrepto-							

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
mycin							
Sulfadimidine	0-50	Full	42 (1994)	100 25 µg/l	Muscle, liver, kidney, fat Milk	Cattle, sheep, pigs, poultry Cattle	Parent drug
Sulphthiazole	No ADI		34 (1989)	No MRL			
Testosterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Tetracycline (see CTC)							
Thiamphenicol	0-6	T	47 (1996)	40 T	Muscle, liver, kidney, fat	Cattle, chickens	Parent drug
Thiabendazole	0-100	Full	48 (1997) 40 (1992)	 100 100 µg/l	 Muscle, liver, kidney, fat Milk	 Cattle, pigs, goats, sheep Cattle, goats	 Sum of thiabendazole and 5-hydroxythiabendazole
Tilmicosin	0-40	Full	47 (1996)	100 1000 1500 300 1000 50 µg/l T	Muscle, fat Liver Liver Kidney Kidney Milk	Cattle, pigs, sheep Cattle, sheep Pigs Cattle, sheep Pigs Sheep	Parent drug
Trenbolone acetate	0-0.02	Full	34 (1989)	2 10	Muscle Liver	Cattle	β-Trenbolone for muscle α-Trenbolone for liver
Triclabendazole	0-3	Full	40 (1992)	200 300 100 100	Muscle Liver, kidney Fat Muscle, liver kidney, fat	Cattle Sheep	5-Chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one
Tylosin	No ADI		38 (1991)	No MRL			
Xylazine	No ADI		47 (1996)	No MRL			
Zeranol	0-0.5	Full	32 (1987)	2 10	Muscle Liver	Cattle	Parent drug

RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

Anthelminthic agents

Eprinomectin

Acceptable daily intake: 0-10 µg/kg bw
Residue definition: Eprinomectin B_{1a}

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Cattle	100	2000	300	250	20

Febantel, fenbendazole, and oxfendazole

Acceptable daily intake: 0-7 µg/kg bw (group ADI for febantel, fenbendazole, and oxfendazole)
Residue definition: Determined as the sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Cattle	100	500	100	100	100
Horses	100	500	100	100	
Pigs	100	500	100	100	
Goats	100	500	100	100	
Sheep	100	500	100	100	100

Moxidectin

Acceptable daily intake: 0-2 µg/kg bw (established at the forty-fifth meeting of the Committee (WHO TRS 864, 1996))
Residue definition: Moxidectin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Cattle[1]	20[2]	100	50	500	
Sheep[1]	50	100	50	500	
Deer	20	100	50	500	

[1]Recommended at the forty-fifth meeting of the Committee (WHO TRS 864, 1996), except for sheep muscle, which was recommended at the forty-seventh meeting (WHO TRS 976, 1998)
[2]At the forty-fifth meeting (WHO TRS 864, 1996), the Committee noted the very high concentration and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.

Antimicrobial agents

Gentamicin

Acceptable daily intake: 0-20 µg/kg bw
Residue definition: Gentamicin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Cattle	100	2000	5000	100	200
Pigs	100	2000	5000	100	

Procaine benzylpenicillin

Acceptable intake: Residues of benzylpenicillin and procaine benzylpenicillin should be kept below 30 µg of penicillin per person per day.
Residue definition: Benzylpenicillin

Recommended maximum residue limits (MRLs)

Species[1]	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Cattle	50	50	50		4
Pigs	50	50	50		
Chickens	50	50	50		

[1]Procaine benzylpenicillin is also used in horses, sheep, turkeys, rabbits, quail, and pheasants. Due to the lack of information, MRLs could not be established for those species.

Sarafloxacin

Acceptable daily intake: 0-0.3 µg/kg bw
Residue definition: Sarafloxacin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Chickens	10	80	80	20	
Turkeys	10	80	80	20	

Spectinomycin

Acceptable daily intake: 0-40 µg/kg bw (established at the forty-second meeting of the Committee (WHO TRS 851, 1995))
Residue definition: Spectinomycin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)	Eggs (µg/kg)
Cattle	500	2000	5000	2000	200	
Pigs	500	2000	5000	2000		
Sheep	500	2000	5000	2000		
Chickens	500	2000	5000	2000		2000

Chlortetracycline, oxytetracycline, and tetracycline

Acceptable daily intake: 0-30 µg/kg bw (group ADI for oxytetracycline, chlortetracycline, and tetracycline)
Residue definition: Parent drug, singly or in combination

Recommended maximum residue limits (MRLs)[1]

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)	Eggs (µg/kg)
Cattle	200	600	1200		100	
Pigs	200	600	1200			
Sheep	200	600	1200		100	
Poultry	200	600	1200			400
Fish[2,3]	200					
Giant prawn[2] (*Penaeus monodon*)	200					

[1]Singly or in combination
[2]Applies only to oxytetracycline
[3]Temporary pending evaluation of use pattern of oxytetracycline in aquaculture

Antiprotozoal agents

Diclazuril

Acceptable daily intake: 0-30 µg/kg bw
Residue definition: Diclazuril

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Sheep	500	3000	2000	1000	
Poultry	500	3000	2000	1000[1]	
Rabbits	500	3000	2000	1000	

[1]Skin/fat

Imidocarb

Acceptable daily intake: 0-10 µg/kg bw
Residue definition: Imidocarb

Recommended maximum residue limits (MRLs)[1]

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Cattle	300	2000	1500	50	50

[1]Temporary. Residue depletion studies in lactating and non-lactating cattle using recommended subcutaneous doses of unlabelled imidocarb and analyzing samples using the proposed regulatory method with enzymatic digestion are required for evaluation in 2001. If MRLs are to be recommended for sheep, a residue depletion study using the recommended dose and route of administration would be required.

Nicarbazin

Acceptable daily intake: 0-400 µg/kg bw
Residue definition: N,N'-bis-(4-nitrophenyl)urea

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat/skin (µg/kg)	Milk (µg/litre)
Chickens (Broilers)	200	200	200	200	

Glucocorticosteroid

Dexamethasone

Acceptable daily intake: 0.015 µg/kg bw (established at the forty-second meeting of the Committee (WHO TRS 851, 1995))

Maximum residue limits: The forty-second and forty-third meetings of the Committee recommended temporary MRLs of 0.5 µg/kg in muscle, 0.5 µg/kg in kidney and 2.5 µg/kg in liver of cattle, horses and pigs and 0.3 µg/L in cattle milk based on an ADI of 0-0.015 µg/kg body weight. The MRLs were temporary because there was no adequate method to determine compliance with the MRLs. The Committee requested performance data on the analytical method for evaluation at its forty-eight meeting but no data were received. The Committee decided to withdraw the temporary MRLs for dexamethasone due to lack of an adequate analytical method for enforcement of the MRLs. The present Committee reviewed the documentation for an HPLC-MS method for measuring dexamethasone residues in tissues and milk. The Committee concluded that the proposed method does not meet the required performance characteristics for identification and quantification of residues in incurred tissues. MRLs could not be recommended because a suitable method for residue analysis was not available.

Production aid

Recombinant bovine somatotropins (rbSTs)

Acceptable daily intake: ADI "not specified"[1] (applies to somagrebove, sometribove, somavubove, and somidobove)

Maximum residue limits: MRLs "not specified" in cattle milk and edible tissues[2] (applies to somagrebove, sometribove, somavubove, and somidobove)

[1]See Annex 1. ADI "not specified" means that available data on the toxicity and intake of the veterinary drug indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that use of the veterinary drug does not represent a hazard to human health and that there is no need to specify a numerical ADI.

[2]See Annex 1. MRL "not specified" means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.

Tranquilizing agent

Azaperone

Acceptable daily intake: 0-6 µg/kg bw
Residue definition: Sum of concentrations of azaperone and azaperol

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Pigs	60	100	100	60	

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This document is one of the three publications prepared by the fiftieth session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome in February 1998 and dedicated exclusively to the evaluation of veterinary drug residues in food. The report of the meeting will be published in the WHO Technical Report Series, and the toxicological monographs as No. 41 in the WHO Food Additives Series. Residue monographs in this document provide information on chemical identity, properties, use, pharmacokinetics, metabolism and tissue residue depletion of, and analytical methods for, substances indicated on the cover. This publication is meant for regulatory authorities, veterinary drug researchers and any other concerned persons who wish to gain information and insights into the needs and problems involved in establishing maximum limits for veterinary drug residues in food.

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